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Contribution to the Chemistry of Oils and Fats With Particular Reference to Butter Fat

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CONTRIBUTION TO THE CHEMISTRY OF OILS AND FATS WITH PARTICULAR REFERENCE TO BUTTER FAT.

Introduction.

In the presentation of a thesis on the chemistry of oils and fats, I am obliged to depart in a measure from the usual form of procedure in such instances, as the work was not undertaken with this object in view and, furthermore, has extended over a considerable period of time. A brief statement of the character of the investigation and of the conditions that initiated the various lines of study seems necessary at the outset in order that the different phases of the subject may be treated as distinct problems.

Experiments in animal nutrition have constituted a prominent line of investigation at the Massachusetts Experiment Station ever since its organization, but not until 1890 was any attempt made by this station to ascertain the effect of feed on the composition of butter fat.[1] The measurement of the effect was limited to determinations of volatile and non-volatile acids by the distillation method of Moore[2] or of Nilson[3]. The latter process represents the extent to which fat analysis was carried at the station at the time of my appointment as assistant. The Association of Official Agricultural Chemists was instrumental in bringing forward other methods and their use gradually became

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[1] Rpts. State Agr. Expt. Sta., 8, pp. 64-69 (1891); 9, pp. 83-86 (1892); 10, pp. 55-56 (1893).

[2] As modified by Waller, Jour. Amer. Chem. Soc., 11, pp. 144-147 (1889).

[3] Ztschr. Analyt. Chem. 28, pp. 175-183 (1889).

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more general. In 1898 a series of feeding experiments was inaugurated by Dr. Lindsey to determine, among other things, the specific effect of the several food groups,--protein, fat and carbohydrates--as found in different feed stuffs, upon the composition of the resulting butter fat.[1] The investigation extended over a number of years and required the analysis of many samples of fat. Having charge of that portion of the analytical work and also of the examination of numerous samples of oleomargarine and of renovated butter for the state Dairy Bureau in connection with its prosecutions, a careful study of the technique of fat analysis, in so far as the more common group methods are concerned, was undertaken at the outset and has been continued to the present time. With increasing experience, plans were formulated for a systematic correlation and standardization of the various processes and considerable progress has been made in that direction. The object was to promote accuracy by greater uniformity and simplicity of methods embracing definition of terms, principles involved and details of manipulation including reagents, apparatus and glassware. A report on "Methods for Fat Analysis"[2] was published some years ago. In the present instance the original features are discussed at some length followed by the revised methods treated in a monographical way. In some cases the modifications are so extensive as to practically constitute new methods, as in Acetyl Number.

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[1] Rpts. Hatch Expt. Sta., 13, pp. 14-33 (1901); 14, pp. 162-168 (1902); 16, pp. 45-62 (1904). Mass. Agr. Expt. Sta. Rpt. 21, Pt. 2, pp. 66-110 (1909).

[2] Rpts. Mass. Agr. Expt. Sta., 21, Pt. 2, pp. 120-138 (1909); 22, Pt. 1, p. 139 (1910).

In the study of butter fat, stability of the product was a factor to be considered. It became necessary to determine whether samples might undergo sufficient chemical change between the time of preparation and of analysis,--several weeks in some instances--as to appreciably modify the results. Therefore experiments were carried out to ascertain the effects of air, light and moisture and also of heat and the results reported as "Stability of Butter-Fat Samples"[1]. The investigation did not prove entirely satisfactory chiefly for the reason that an opaque, solid fat was ill adapted for the purpose, as the action of the several agents was restricted in a large measure to the surface of the fat, extending very slowly into the mass. Consequently another experiment of similar character was instituted under better control conditions, to determine the stability of olive oil, in hopes that the facts secured might prove of general application, and of practical value. Five years of the test will be completed in 1915, but not in season to be included in this paper; sufficient samples will still remain for another year.

Upon the discontinuance of the series of feeding experiments to which reference has been made, it was evident that while the old "group" methods of fat analysis were very serviceable, furnishing valuable information relative to the nature and quality of the product, they were insufficient for a technical investigation such as was undertaken. As fresh butter fat is composed largely of triglycerides, a determination of the different fatty acids would provide a better criterion of the effect of food constituents. To be sure, some differentiation in fatty acids was possible by the methods in vogue, but the results as a whole were indicative rather than determinate. Therefore an attempt was made to perfect practical methods for the determination of the various fatty acids, particularly the insoluble acids as they constitute from 86.50 to 90 percent of the fat. In pursuance of this work, a supply of high grade fatty acids was required and as the best obtainable on the market proved unsatisfactory, their purification became a problem of considerable importance. Three different methods for purifying saturated acids were investigated and their merits and disadvantages summarized under "Purification of Insoluble Fatty Acids"[1]. Subsequently attention was directed to the treatment of oleic acid, the most prominent unsaturated acid of edible oils and fats, and a fair measure of success obtained.

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[1] Mass. Agr. Expt. Sta. Rpt. 22, Pt. 1, pp. 132-138 (1910).

After securing a stock of lauric, myristic, palmitic, stearic and oleic acids, the object of the investigation was to adapt or devise methods for the quantitative determination of the different acids in mixtures such as the insoluble acids of butter fat. A separation of that character has been recognized for a long time as one of the most difficult problems of technical chemistry. The Partheil and Ferie method[2] was adopted as a tentative plan of operation as it seemed the most promising. The work was continued several years except during hot weather and the usual interruptions of laboratory practice. The process, however, failed to yield trustworthy results after long trial and many attempts at remedial measures and, in our hands at least, proved entirely impracticable. The investigation, which will be described briefly, although unsuccessful in the main, served to bring to light a mass of valuable information relative to solutions and details of manipulation that have since been found of great assistance.

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[1] Mass. Agr. Expt. Sta. Rpt. 23, Pt. 1, pp. 131-134 (1911).
[2] Arch. Pharm. 241, pp. 545-569 (1903).

A method for determining stearic acid was devised by Hehner and Mitchell[1] that yielded considerable amounts from the insoluble acids of various products but showed no appreciable separation from the acids of butter fat. Believing that an accurate determination of stearic acid in addition to that of oleic acid might warrant, in case of butter, a reasonable inference as to the residual acids, if homologous, and possibly their calculation, if adjacent, was sufficient to induce a thorough study of the process, the results of which are presented as "Determination of Stearic Acid in Butter Fat".

Attention has been directed to the various lines of investigation and the subject matter will be treated under the following distinct headings:

1. Improved Methods for Fat Analysis.
2. Stability of Butter Fat Samples.
3. Purification of Insoluble Fatty Acids and Purification of Oleic Acid.
4. An Attempt at Separating Fatty Acids by their Lithium Salts.
5. Determination of Stearic Acid in Butter Fat.

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[1] Analyst, 21, pp. 316-331 (1896).

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1. IMPROVED METHODS FOR FAT ANALYSIS.

Before considering methods, oils, fats and waxes are defined, classified, and a synopsis of composition given in order that the value of the data contributed by the various determinations whereby the "structural" composition of the product is evolved may be fully understood. The organoleptic tests are merely enumerated, as their application is self-evident. They are employed in classification, are very serviceable in identification, and particularly valuable in discriminating as to quality or grade, for which experience and general knowledge of the trade are essential.

The more prominent physical tests are of such a well-known character that time will not be taken to consider their special modifications. They furnish a certain amount of confirmatory evidence and are occasionally employed for "culling" suspicious samples. With lubricating and illuminating (hydrocarbon) oils they are far more important. The chemical methods are indispensable for determining the identity, composition and quality of oils and fats.

Attention is directed to some of the changes introduced as a result of our work at the experiment station.

Apparatus.--In correlating the various methods for the analysis of oils and fats, apparatus was one of the first subjects that had to be considered. There was a wide diversity of opinion expressed by different writers as to character, form, and size of the container for the several determinations. While a single flask might not be equally convenient in all cases, practical manipulation required its adoption, if feasible, and a 300 c.c. Erlenmeyer flask of uniform height and cork requirement has proved very satisfactory for the purpose. At that time American manufacturers did not produce the desired article and a special mold had to be procured. Graduated ware as a whole was on a rather indefinite basis although the Mohr c.c. was the prevailing system. Normal graduated ware on the basis of true c.c. at 4°C was adopted as the standard, however, and is gradually becoming universal. Flasks and cylinders are graduated for capacity and burettes and pipettes for delivery at 20°C.

For drying insoluble acids, unsaponifiable matter, etc., requiring constant temperature and a continuous circulation of air or inert gas, three ovens of different types have been constructed, namely, a glycerine and water oven heated by gas, a jacketed vacuum oven heated by gas or steam, and an electric ovën automatically controlled by a thermostatic relay. All were found reasonably satisfactory but the electric oven on account of its rapid drying with a comparatively small amount of decomposition, proved the most desirable. A special water bath with false bottom, movable water level, interchangeable tops and superstructure, heated by gas or steam, was constructed for the determination of acid, saponification, and acetyl numbers, insoluble acids, etc., and found indispensable for such work. The superstructure permits the use of glass spiral condensers without danger of breakage.

A double filter bath for removing curd, dirt, water and other impurities from melted fat has demonstrated its value by years of service.

Glass beads, weighing about .5 gram each, have been found one of the best agents to promote ebullition in the determination of acid and saponification numbers, and to expell volatile acids in the Reichert-Meissl test.

Reagents.--The occurrence of aldehyde in practically all alcohol shipped in wood, together with more or less acid, is objectionable for fat analysis. The aldehyde in the presence of caustic alkali imparts a dark reddish brown color to the solution, which obscures the end point in titration when phenolphthalein is used as indicator. After numerous experiments with potassium hydroxide, caustic lime and silver nitrate, a simple and efficient method of purification was devised: Five gallons of alcohol are treated with at least 10 grams of powdered silver nitrate, thoroughly shaken, and allowed to stand several days to insure oxidation of the aldehyde ; then, with 500 grams of calcium oxide (caustic), repeatedly shaken for several days to remove acids and a portion of the water, filtered, and redistilled. Fractionation is necessary if the boiling point exceeds a range of 1°C.

For the preparation of alcoholic potash, the dry product should never be employed as the intensity of the reaction almost invariably results in the formation of aldehyde even with the best grade of alcohol. Fifty c.c. of a saturated solution of potassium hydroxide slowly added, with agitation, to 1000 c.c. of cold alcohol yield a solution approximately .65 normal and free from color.

The advantages of glycerol over alcohol as a carrier of alkali in saponification for the determination of volatile and insoluble acids are so evident that they do not warrant discussion. The substitution of potassium hydroxide in place of sodium hydroxide, however, appears to have been overlooked by most analysts. The potassium compound has less basicity, per gram but the resulting soap is more readily soluble and easier to handle. One hundred and twenty grams of stick potash dissolved in 1000 c.c. of pure glycerol by heating to 105°C yield a very efficient solution of about 1.6 normality.

Hydrochloric acid having shown a tendency to be more destructive than sulfuric acid in the determination of insoluble acids, the latter has been employed exclusively in recent years in the least possible excess consistent with a clear separation of fatty acids and underlying liquid. Some loss, however, is practically unavoidable.

The limitations of phenolphthalein in the presence of aldehydes and in the titration of acid number led to a study of other indicators. Alkali blue has been occasionally mentioned by foreign writers but apparently seldom employed. Of the two alkali blues listed, 6B proved superior to 2 OLA. 6B was somewhat soluble in water but more so in alcohol. Tinctures of the original material, of the water-extracted residue and of the evaporated water extract were tested and a solution, prepared by extracting 1 gram of the original with boiling 95 percent alcohol under a reflux and filtered was found preferable.

The indicator is blue with acids and red with caustic alkalies and only applicable to alcoholic solutions. Alkali blue is sensitive and with experienced workers gives results similar to phenolphthalein in titration of acid and saponification numbers. It has some advantages and may supplement but probably never supersede the latter indicator.

Starch paste is one of the most delicate indicators in use but many fail to recognize that complete colloidization is necessary for the attainment of a true end point, as solid particles react more slowly than a solution.

The strength, method of preparation and quantity of reagents employed have been modified in some instances which will not be considered.

Method.--In standardizing the methods an effort was made to use not only the same flasks but also the same amount of material, volume of solution and indicator, and the same agent to facilitate boiling and like conditions of treatment in so far as possible. All the methods, practically without exception, have been modified in reagents and manipulation and attention called to numerous precautions found necessary for accurate work. This is especially noticeable in the determination of insoluble acids, iodine number and neutralization number of insoluble acids. The acetyl number should be considered a new method. The limits of error are original, based chiefly on practical manipulation, although considered on theoretical grounds. The synopsis of reaction expresses the successive steps and underlying principle in each process free from verbiage. The supplementary notes include any information, original or otherwise, that might be of service in interpreting results. All tables and formulas are calculated on the latest atomic weights and many formulas express old principles in a new light.

CLASSIFICATION.

Natural oils may be divided into two major groups, i.e., essential, ethereal or volatile oils, and fatty, fixed or nonvolatile oils.

The fatty oils may be subdivided according to consistency at ordinary temperature into oils and fats; according to source, into vegetable and animal; according to properties, into drying, semidrying, and nondrying, etc.

Waxes are generally grouped with the fatty oils on account of their similar chemical structure. Oils and fats are essentially neutral glyceryl esters, compounds of fatty acids and the soluble tribasic alcohol, glycerol. Waxes are composed of esters of fatty acids and insoluble monobasic and dibasic alcohols together with a considerable proportion of free alcohols and of hydrocarbons.

Any general classification of oils, fats, and waxes whether of origin, of physical characteristics, or of chemical characteristics is open to criticism; probably that of Lewkowitsch based on the magnitude of the iodine number correlated with that of consistency, origin, and properties is the best that has been offered.

OILS, FATS AND WAXES.

Oils	Vegetable oils	Drying oils	
		Semidrying oils	Cottonseed oil group --- Rape oil group
		Nondrying oils	--- Castor oil group
	Animal oils	Marine animal oils	Fish oils Liver oils Blubber oils
		Terrestrial animal oils	
Fats	Vegetable fats		Chaulmoogra oil group Laurel oil group Palm oil group Myristica group Cacao butter group Coconut oil group Dika fat group ---
	Animal fats	Body fats Drying fats Semidrying fats Nondrying fats Milk fats	
Waxes	Liquid waxes	Animal waxes	
	Solid waxes	Vegetable waxes Animal waxes	

ORGANOLEPTIC TESTS.

Consistency (20°C.)	Liquid Semifluid Solid
Turbidity	Water or other nonmiscible substances
Sediment	Stearin, dirt, etc.
Color[1]	Water white (colorless) Straw color Lemon yellow Bright yellow (most oils when refined) Dirty yellow (beeswax) Bright red Dirty dark red (crude palm oil) Yellowish green (laurel oil) Green (chlorophyll) Brown Black
Opacity	
Fluorescence or bloom	Mineral oils
Odor (cold and hot)	Characteristic of product Rancidity
Taste	Characteristic of product Finest (first quality), "hard", "harsh" Rancidity

[1] Arbitrary commercial terms are often employed in designating oils. A scientific color standard of the Milton Bradley Company's type appears impractical, but Lovibond's tintometer promises more satisfactory results.

PHYSICAL TESTS.

Specific gravity	$d\frac{t}{4^\circ}$ C. $t = 20^\circ$, 40° and 60° C.
Melting point	°C.
Solidifying point	°C.
Refractive index	Abbe refractometer, $n\frac{t}{D}$ $t = 20^\circ$, 40° and 60° C.
Optical rotation	Dextro rotatory 200 m.m. tube Laevo rotatory
Colorimeter	Lovibond tintometer
Viscosity	Redwood viscosimeter 70° Fahr.
Solubility	Crismer, Critical Temperature of Dissolution Valenta test
Flash point	°C.
Ignition point	°C.

SYNOPSIS OF COMPOSITION--OILS AND FATS.

Neutral fat	Fatty acids
	Glycerol
Free fatty acids	
Unsaponifiable matter[1]	Sterols[2]
	Hydrocarbons
	Chromogenic bodies, resinous substances, etc.

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[1] Nominally a part of the neutral fat but differentiated to facilitate subsequent calculations.

[2] Occur as free alcohols and, to some extent, in combination with fatty acids as esters.

FATTY ACIDS AND GLYCERIDES.

Total Fatty Acids.

Neutralization number	
Mean molecular weight	
Fatty Acids of Neutral Fat.	Glycerides. (Neutral Fat).
Neutralization number	Saponification number
Mean molecular weight	Mean molecular weight
Glycerol	
Free Fatty Acids.	
Neutralization number	
Mean molecular weight	
Soluble Fatty Acids.[1]	Glycerides.
Neutralization number	Saponification number
Mean molecular weight	Mean molecular weight
Acetic acid	Acetin
Butyric acid	Butyrin
Valeric acid	Valerin
Caproic acid	Caproin
Caprylic acid	Caprylin
Capric acid	Caprin
Glycerol	
Volatile Fatty Acids.[2]	Glycerides.
Neutralization number	Saponification number
Mean molecular weight	Mean molecular weight
Glycerol	
Insoluble Fatty Acids.[1]	Glycerides.
Neutralization number	Saponification number
Mean molecular weight	Mean molecular weight
Lauric acid	Laurin
Myristic acid	Myristin
Palmitic acid	Palmitin
Stearic acid	Stearin
Arachic acid	Arachin
Oleic acid	Olein
Erucic acid	Erucin
Linolic acid	Linolin
Linolenic acid	Linolenin
Clupanodonic acid	Clupanodonin
Ricinoleic acid	Ricinolein
Dihydroxystearic acid	Dihydroxystearin
Glycerol	

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[1] The free soluble and insoluble acids should not be calculated to glycerides.

[2] Are considered as constituting a portion of the soluble acids.

SAPONIFICATION (KOETTSTORFER[1]) NUMBER.

The saponification number indicates the milligrams of potassium hydroxide required for the complete saponification of 1 gram of an oil, fat or wax.

Reagents.--Alcohol[2], redistilled, free from acids and aldehydes.

Alcoholic potash solution[3], 50 c.c. of a saturated solution of potassium hydroxide, free from carbonate, to 1000 c.c. of alcohol. The alkali should be added to the alcohol slowly with agitation in order to prevent any appreciable rise in temperature. The solution should be allowed to stand at least 24 hours and filtered immediately before use.

N/2 hydrochloric acid.

Phenolphthalein solution, 1 gram to 100 c.c. of alcohol, neutralized.

Alkali blue (6B) solution, 1 gram to 100 c.c. of alcohol. The indicator should be boiled in a flask under a reflux condenser for 2 hours and then filtered.

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[1] Ztschr. Analyt. Chem., 18, pp. 199-207, 431-437, (1879).

[2] All alcohol used as a solvent in fat analysis or in preparation of the reagents should be treated with at least 10 grams of powdered silver nitrate to 5 gallons of alcohol, thoroughly shaken, and allowed to stand for 2 days to insure oxidation of the aldehydes; then with 500 grams of dry calcium oxide for several days, repeatedly shaken, filtered and redistilled. This insures the removal of acids, aldehydes and a portion of the water. In many instances it has been found necessary to fractionate the alcohol for substances of low boiling points as well as esters frequently occur. This is particularly true of alcohol that is recovered from laboratory processes. The distillate should be preserved in glass and protected from sunlight.

[3] Approximately .65N solution. Alcoholic potash will dissolve about .04 grams of potassium carbonate to the 100 c.c. according to Holde (Chem. Rev. Fett u. Harz Indus., 14, pp. 105-107 (1907).

Method.--Into a 300 c.c. Erlenmeyer flask are brought 5 grams of fat, care being taken to avoid getting any fat on the sides of the flask, together with 50 c.c. of alcoholic potash[1], accurately measured with a burette, 50 c.c. of alcohol and several glass beads. The flask is connected with a spiral or other form of reflux condenser and the solution boiled on a water bath with occasional rotating of the contents, until saponification is complete, about 60 minutes. The flask is then placed in a water bath at 60° C. and the solution, after cooling to that temperature, titrated with N/2 hydrochloric acid using 1 c.c. of phenolphthalein as indicator, to the complete elimination of the pink color. The cooling and dilution of the solvent due to the addition of the acid occasionally give rise to small, colored particles; this can be obviated by a slight increase in temperature. The end point is particularly difficult to determine in the presence of aldehydes which impart a dark reddish-brown color to the solution. In such cases alkali blue (1 c.c.) is preferable to phenolphthalein. The change in color is from red with alkalies to blue with acids. The two indicators appear to give like results when considered in connection with their respective blanks. Absorption of carbonic acid from the air should be guarded against at all times. As alcoholic potash gradually loses alkalinity on boiling, the operation should be timed with reasonable care.

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[1] For waxes, especially wool wax, potassium alcoholate is preferable on account of its greater efficiency. The solution should be freshly prepared by dissolving 5 grams of metallic potassium in 100 c.c. of absolute alcohol.--Lewkowitsch, Analysis of Oils, Fats and Waxes, 1, p. 107 (1913).

Several blank determinations should be run with every series of tests under precisely similar conditions. The difference between the titration of the blank and that of the excess alkali in the test is the acid equivalent of the fat taken which should be calculated to milligrams of potassium hydroxide for 1 gram of fat.

One c.c. of N/2 acid is equivalent to 28.054 milligrams of potassium hydroxide.

Limit of error, 0.50 saponification number.

Synopsis of Reaction.--

$$\underset{\text{fat}}{(RCOO)_3C_3H_5} + \underset{\text{alkali}}{3KOH} = \underset{\text{salt}^1}{3RCOOK} + \underset{\text{glycerol}}{C_3H_5(OH)_3}$$

Titration of excess alkali

R in the graphic formula of the fatty acids represents C and H in different amounts, according to the acid, but in the proportion of C_nH_{2n+1}, except in the case of unsaturated acids.

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[1] The term "soap" is now limited by custom to the alkali salts of insoluble fatty acids.

Saponification Numbers of Triglycerides.

Glyceride.	Formula.	Molecular Weight.	Saponification Number.
Acetin	$(CH_3COO)_3C_3H_5$	218.112	771.732
Butyrin	$(C_3H_7COO)_3C_3H_5$	302.208	556.981
Valerin	$(C_4H_9COO)_3C_3H_5$	344.256	488.950
Caproin	$(C_5H_{11}COO)_3C_3H_5$	386.304	435.729
Caprylin	$(C_7H_{15}COO)_3C_3H_5$	470.400	357.832
Caprin	$(C_9H_{19}COO)_3C_3H_5$	554.496	303.562
Laurin	$(C_{11}H_{23}COO)_3C_3H_5$	638.592	263.586
Myristin	$(C_{13}H_{27}COO)_3C_3H_5$	722.688	232.914
Palmitin	$(C_{15}H_{31}COO)_3C_3H_5$	806.784	208.636
Stearin	$(C_{17}H_{35}COO)_3C_3H_5$	890.880	188.941
Arachin	$(C_{19}H_{39}COO)_3C_3H_5$	974.976	172.644
Olein	$(C_{17}H_{33}COO)_3C_3H_5$	884.832	190.233
Erucin	$(C_{21}H_{41}COO)_3C_3H_5$	1053.024	159.848
Linolin	$(C_{17}H_{31}COO)_3C_3H_5$	878.784	191.542
Linolenin . . .	$(C_{17}H_{29}COO)_3C_3H_5$	872.736	192.869
Clupanodonin . .	$(C_{17}H_{27}COO)_3C_3H_5$	866.688	194.215
Ricinolein . . .	$(C_{17}H_{32}.OH.COO)_3C_3H_5$	932.832	180.444
Dihydroxystearin	$(C_{17}H_{33}(OH)_2COO)_3C_3H_5$	986.880	170.562

Supplementary Notes.--The term "saponification or saturation equivalent", as employed by Allen[1] and others, indicates the grams of fat that are saponifiable with one equivalent of potassium hydroxide in grams (56.108); in other words, the grams of fat saponifiable with 1 liter of N/1 potassium hydroxide.

$$\text{Saponification equivalent (sq)} = \frac{56108}{s} \text{ or } \frac{\text{mg. of fat}}{\text{c.c. N/1 alkali}}$$

$$\text{Saponification number (s)} = \frac{56108}{sq}$$

The lower the molecular weight of the fatty acids (or esters) the more alkali will be required to satisfy 1 gram, and the higher will be the saponification number. The presence of free fatty acids increases the saponification number and unsaponifiable matter decreases it. The majority of oils and fats have saponification numbers lying between 185 and 200 with a mean of approximately 193. While the numbers for different products are quite characteristic they are by no means fixed or constant, varying with the state of purity and rancidity. The character of an oil or fat seems to be affected also by natural conditions such as climate, soil, food supply and other factors influencing formation or production, and to some extent by method of separation or preparation.

Fats and oils containing a considerable amount of the glycerides of the lower (volatile) fatty acids and of myristin are characterized by a saponification number exceeding 200. Among the more prominent of these, testing from 210 to 290, are croton oil, spindle tree oil, turtle, dolphin jaw, porpoise jaw and brown fish of the blubber oils, several of the myristica group, the coconut oil group including palm-nut, coconut and other less common oils, the Dika fat group, Japan wax and butter fat. Oils and fats containing a considerable proportion of glycerides of the higher fatty acids, particularly hydroxy acids, are characterized by low saponification numbers. Castor oil, consisting largely of ricinolein, has a saponification number of about 185. The rape oil group including rape (colza) and various mustard oils have saponification numbers of about 175, on account of the large proportion of erucin.

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[1] Commercial Organic Analysis, 2, pp. 15-16 (1910).

Liquid and solid waxes such as sperm oil, flax wax, wool wax, beeswax, spermaceti, insect wax, etc., are characterized by extremely low saponification numbers, from 80 to 140, due to the large proportion (about one-half) of monobasic alcohols and of hydrocarbons.

Monoglycerides, with only one acid radical, and diglycerides, with two, have a lower saponification number than the corresponding triglycerides with three acid radicals.

ACID NUMBER.

The acid number indicates the milligrams of potassium hydroxide required to neutralize the free fatty acids in 1 gram of an oil, fat or wax.

Reagents.--Alcohol, redistilled, free from acids and aldehydes.

N/10 potassium (or sodium) hydroxide solution.

Phenolphthalein solution, 1 gram to 100 c.c. of alcohol, neutralized.

Alkali blue (6B) solution, 1 gram to 100 c.c. of alcohol. The indicator should be boiled in a flask under a reflux condenser for 2 hours and then filtered.

Method.--Ten grams of fat are brought into a 300 c.c. Erlenmeyer flask together with 100 c.c. of alcohol and several glass beads. The flask is connected with a spiral or other form of reflux condenser and the solution brought to boil on a water bath to insure solution of the free fatty acids. The boiling should not be prolonged as esterification is likely to result. The flask is then placed in a water bath at 60° C. and the solution, after cooling to that temperature, titrated with N/10 alkali, using 1 c.c. of phenolphthalein as indicator, to the appearance of a pink tint. N/2 alkali is preferable for high percentages of free acids, preventing unnecessary dilution and cooling of the solvent which otherwise might cause partial hydrolysis of the resulting soap[1] if the alcoholic strength fell below 40 percent[2]. In practical work the minimum strength should be 50 percent to insure safety. As the change in color with phenolphthalein is gradual, in many instances without a sharply defined end point, alkali blue (1 c.c.) offers certain advantages in such cases as it yields a pronounced red and is more decisive. The coloration is not permanent with either indicator because of the saponification of neutral esters and the decolorizing action of carbonic acid absorbed from the air on shaking. Thorough shaking during titration, however, is essential although the color persists for only a short time.

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[1] On diluting a solution of neutral alkali palmitate or stearate (RCOOK), a salt containing more than one equivalent of fatty acids to one of alkali is produced, and the ratio tends to increase with greater dilution. Neutral oleate, however, requires a large quantity of water and low temperature before it will hydrolize.

[2] Kanitz, Ber. Deut. Chem. Gesell., 1903, p. 400. (From Lewkowitsch).

Several blank determinations should be run on the alcohol with every series of tests and deducted. Redistilled alcohol should be practically neutral or can be made so readily by the addition of alkali.

One c.c. N/10 alkali is equivalent to 5.6108 milligrams of potassium hydroxide.

Limit of error, 0.10 acid number.

Synopsis of Reaction.--

$$RCOOH + KOH = RCOOK + H_2O$$

Supplementary Notes.--Koettstorfer expresses the acidity in cubic centimeters of N/1 potassium hydroxide required for 100 grams of fat as "degrees of acidity". Stockmeier[1] reports "degrees of rancidity" in the same manner. N/10 alkali and 10 grams of fat are, however, more convenient amounts with which to work.

1° rancidity = .56108 acid number.

1 acid number = 1.78228° rancidity.

The amount of free acids in lubricating oils is sometimes reported in terms of sulfuric anhydride (SO_3).

The acid number of oils and fats varies with the purity, age, and the amount of hydrolysis and of oxidation they have undergone. Contact with fermenting or decaying matter such as animal tissue, casein of butter and the marc of fruits tends to rapidly increase the amount. Acidity is not a measure of rancidity, as hydrolysis may result from the action of enzymes in the presence of moisture without accompanying oxidation which appears necessary for the production of strong-smelling, acrid-tasting bodies that characterize rancid products. Rancidity is apparently due to the simultaneous action of oxygen and of light on free fatty acids. Rancidity develops more readily in liquid oils in which olein predominates than in the solid fats which are composed more largely of palmitin and stearin. Fresh animal fats are practically free from acid, while vegetable oils seem to contain a small amount. Relatively large amounts of free fatty acids are sometimes found in corn, sesame, peanut, rice, olive (especially "bagasse" olive oils), and Japanese sardine oils, in the so-called vegetable butters and tallows and other vegetable fats particularly palm oil, and in bone fat, beef tallow and butter. The amount of free fatty acids in waxes is probably smaller and the variation less than in oils and fats, although carnauba wax and especially beeswax appear to contain considerable. In a way, acid number indicates "quality" of the product.

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[1] Abstract, Vrtljschr. Chem. Nahr. u. Genussmtl. 4, pp. 428-429, (1889).

Mineral acids when present may be determined by shaking out with hot water in a separatory funnel and by titrating the solution when cold with N/2 or N/10 alkali (according to the amount of acids present), using 1 c.c. of methyl orange (1-1000) as indicator. Methyl orange is not affected by carbonic acid, by the insoluble fatty acids, and only to a limited extent by the soluble fatty acids, and is, therefore, well adapted for the purpose.

ESTER (ETHER) NUMBER.

The ether number indicates the milligrams of potassium hydroxide required for the saponification of the neutral esters in 1 gram of an oil, fat or wax.

The ether number is represented by the difference between the saponification and acid numbers, and in cases where there are no free fatty acids present, is identical with the saponification number.

Supplementary Notes.-- Natural fats, both animal and vegetable, contain practically only triglycerides,--neutral glyceryl esters. These glycerides may occur, however, to a considerable extent as complex molecules (mixed triglycerides) instead of simple. The composition of mixed glycerides is difficult to determine as they appear to suffer intramolecular changes on being treated with a solvent.

Monoglycerides and diglycerides apparently never occur in nature or to any apprecialbe amount in freshly prepared oils and fats. Lewkowitsch asserts that the presence of free fatty acids indicates previous hydrolysis of the triglycerides, and hydrolysis conditions the presence of monoglycerides and diglycerides; therefore, the so-called ether number loses its definite character as free acids increase.

CALCULATED DATA FROM SAPONIFICATION, ACID AND ETHER NUMBERS.

Glycerol.--In the saponification of any triglyceride, 3 molecules or 168.324 parts of potassium hydroxide combine with 1 molecule of fat, setting free 1 molecule or 92.064 parts of glycerol; therefore 1 gram of potassium hydroxide is equivalent to .54695 gram of glycerol.

$$\underset{\text{fat}}{(RCOO)_3C_3H_5} + \underset{\text{alkali}}{3KOH} = \underset{\text{glycerol}}{C_3H_5(OH)_3} + \underset{\text{salt}}{3RCOOK}$$

The percentage of glycerol (G) can be calculated from the ether number (e) by means of the formula[1]:

$$G = .00054695\ e \qquad (1)$$

This formula is not applicable in the case of fats containing monoglycerides and diglycerides. The higher the saponification number or, in other words, the lower the mean molecular weight of the constituent acids, the greater the proportion of glycerol. Monoglycerides and diglycerides contain a larger proportion of glycerol than the triglycerides.

Total Fatty Acids.--In the saponification of a fat, 3 molecules or 54.048 parts of water are required for every molecule or 92.064 parts of the glycerol separated.

$$\underset{-}{(RCOO)_3C_3H_5} + \underset{54.048}{3H_2O} = \underset{92.064}{C_3H_5(OH)_3} + \underset{-}{3RCOOH.}$$

The percentage of total fatty acids (T) in 1.00 part of fat can be calculated from the percentage of glycerol (G) by means of the formula[2]

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[1] Zulkowski, Ber. Deut. Chem. Gesell. 16, p. 1140.
[2] Loco citato, p. 1315.

$$T = 1.00 + \frac{54.048}{92.064} G - G \quad \text{or}$$

$$1.00 - \frac{38.016}{92.064} G$$

and substituting the value of glycerol in terms of ether number (e):

$$T = 1.00 - \left(\frac{38.016}{92.064} \times .00054695\ e\right) \quad \text{or}$$

$$1.00 - .00022585\ e \qquad (2)$$

In other words, assuming a fat to be composed of a mixture of triglycerides and free fatty acids, if the group C_3H_2 in an amount proportional to the glycerol content be deducted from 1.00, the percent of total fatty acids may be obtained.

$$(RCOO)_3C_3H_5 + RCOOH - C_3H_2 = 4RCOOH$$

$$T = 1.00 - \frac{38.016}{92.064} G \quad \text{or}$$

$$1.00 - .00022585\ e \qquad (2)$$

In case of fats containing appreciable amounts of unsaponifiable matter, proper correction should be made for the same.

Neutralization Number and Mean Molecular Weight of Total Fatty Acids.--The neutralization number (n) and mean molecular weight (m) of the total fatty acids (T) can be calculated from the ether (e) and saponification (s) numbers by means of the formulas:

$$n = \frac{s}{T} \quad \text{or}$$

$$\frac{s}{1.00 - .00022585\ e} \qquad (3)$$

$$m = \frac{56108}{n} \qquad (4)$$

Neutralization Number and Mean Molecular Weight of Fatty Acids in Neutral Fat.--The molecular weight (m_1) and neutralization number (n_1) of the acids in the neutral fat can be calculated from the ether number (e) and the percentage of neutral fat (F)--determined either gravimetrically or by difference[1]--by the formulas[2]:

$$\text{Molecular Weight of Neutral Fat} = \frac{3 \times 56108}{e} \times F \quad \text{or}$$

$$\frac{168324\ F}{e}$$

$$\text{Molecular Weight of Neutral Fat} = 3\ (m_1 - H) + C_3H_5 \quad \text{or}$$

$$3\ (m_1 - 1.008) + 41.04 \quad \text{or}$$

$$3m_1 + 38.016$$

$$3m_1 + 38.016 = \frac{168324\ F}{e}$$

$$m_1 = \frac{56108\ F}{e} - 12.672 \qquad (5)$$

$$n_1 = \frac{56108}{m_1} \qquad (6)$$

The neutralization number (n_1) and molecular weight (m_1) of the acids in the neutral fat can also be calculated from the ether number (e) and the percentage of total fatty acids (T) and free fatty acids (A) by the formula:

$$n_1 = \frac{e}{T - A} \qquad (7)$$

$$m_1 = \frac{56108}{n_1} \qquad (8)$$

Fatty Acids and Glycerol in Neutral Fat.--The fatty acids (N) and glycerol (G) in neutral fat can be calculated from the percentage of neutral fat (f) and the mean molecular weight (m_1) of the fatty acids in the neutral fat[1]:

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[1] The unsaponifiable matter is a source of error unless deducted.

[2] Wright, Analysis of Oils, etc., p. 130 (1903).

$$N = \frac{3m_1}{3m_1 + C_3H_2} \times F \qquad (9)$$

$$G = \frac{92.064}{3m_1 + 38.016} \times F \qquad (10)$$

The amount of glycerol (G) required for combination with the fatty acids to form neutral fat (F) can also be calculated from the amount (N) and molecular weight (m_1) of the acids of neutral fat by the formula:

$$F = \frac{3m_1 + C_3H_2}{3m_1} \times N \text{ or}$$

$$\frac{3m_1 + 38.016}{3m_1} \times N$$

$$G = \frac{C_3H_5(OH)_3}{3m_1 + C_3H_2} \times F \text{ or}$$

$$\frac{92.064}{3m_1 + 38.016} \times F$$

Substituting the value of F in terms of m_1 and N

$$G = \frac{92.064}{3m_1 + 38.016} \times \frac{3m_1 + 38.016}{3m_1} \times N$$

$$= \frac{92.064}{3m_1} \times N \qquad (11)$$

The total fatty acids are equal to the sum of the fatty acids in the neutral fat and the free fatty acids.

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[1] A close approximation can usually be obtained by using the mean molecular weight of the total fatty acids.

Percentage of Fatty Acids and Glycerol in Triglycerides.

Glyceride.	Formula.	Fatty Acids. (Per Cent).	Glycerol. (Per Cent).
Acetin	$(CH_3COO)_3C_3H_5$	82.570	42.210
Butyrin	$(C_3H_7COO)_3C_3H_5$	87.421	30.464
Valerin	$(C_4H_9COO)_3C_3H_5$	88.957	26.743
Caproin	$(C_5H_{11}COO)_3C_3H_5$	90.159	23.832
Caprylin	$(C_7H_{15}COO)_3C_3H_5$	91.918	19.571
Caprin	$(C_9H_{19}COO)_3C_3H_5$	93.144	16.603
Laurin	$(C_{11}H_{23}COO)_3C_3H_5$	94.047	14.417
Myristin	$(C_{13}H_{27}COO)_3C_3H_5$	94.740	12.739
Palmitin	$(C_{15}H_{31}COO)_3C_3H_5$	95.288	11.411
Stearin	$(C_{17}H_{35}COO)_3C_3H_5$	95.733	10.334
Arachin	$(C_{19}H_{39}COO)_3C_3H_5$	96.101	9.443
Olein	$(C_{17}H_{33}COO)_3C_3H_5$	95.704	10.405
Erucin	$(C_{21}H_{41}COO)_3C_3H_5$	96.390	8.743
Linolin	$(C_{17}H_{31}COO)_3C_3H_5$	95.674	10.476
Linolenin	$(C_{17}H_{29}COO)_3C_3H_5$	95.644	10.549
Clupanodonin	$(C_{17}H_{27}COO)_3C_3H_5$	95.614	10.623
Ricinolein	$(C_{17}H_{32}.OH.COO)_3C_3H_5$	95.925	9.869
Dihydroxystearin	$(C_{17}H_{33}(OH)_2COO)_3C_3H_5$	96.148	9.329

Free Fatty Acids.--The acid number (a) can be readily converted into percentage of free fatty acids (A) expressed as oleic, as sulfuric anhydride (SO_3), as an assumed acid with a molecular weight determined by formula (4), or as the acid of any other molecular weight (m_2).

$$A = \frac{a \times m_2}{56108} \qquad (12)$$

When the free acid or the predominant acid in a mixture is known, it is often desirable to report acidity in terms of that acid. In such cases it is preferable to calculate the percentage directly from the titration by factot .0001 of the molecular weight of the acid (monobasic) for an N/10 solution, or .001 for N/1.

The percent of acidity (A) can also be calculated from the acid number (a) and the neutralization number (n) of the total fatty acids[1].

$$A = \frac{a}{n} \qquad (13)$$

The amount of free fatty acids can be estimated approximately from the acid number (a) and saponification number (s):

$$A = \frac{a}{s}$$

Neutral Fat and Unsaponifiable Matter.--The neutral fat and unsaponifiable matter can be determined by difference--1.00 minus the percent of free fatty acids.

Neutral Fat and Free Fatty Acids.--The amount of neutral fat (F) and free fatty acids (A) can be estimated from the ether number (e) by assuming an average saponification number (s_x) for the neutral product as basis for the calculation.

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[1] Strictly it should be the neutralization number of the free fatty acids. The number of cubic centimeters of N/1 alkali required can be substituted in place of the values a and n.

$$F = \frac{e}{s_x}$$

$$A = 1.00 - F.$$

Mean Molecular Weight and Saponification Number of Neutral Fat.--The molecular weight (m_3) of the amount of neutral fat can be calculated from the ether number (e) and the amount of neutral fat (F) by the formula:

$$m_3 = \frac{3 \times 56108}{e} \times F \qquad (14)$$

or from the mean molecular weight (m_1) of the acids of the neutral fat:

$$m_3 = 3m_1 + 38.016 \qquad (15)$$

See derivation of formula (5).

The sapponification number (s_3) can be calculated from the mean molecular weight (m_3) by means of the formula:

$$s_3 = \frac{3 \times 56108}{m_3} \qquad (16)$$

SOLUBLE FATTY ACIDS.

The soluble fatty acids indicate the percentage of fatty acids in an oil, fat or wax, that is soluble in water[1].

The percentage of soluble fatty acids can be readily calculated by difference,--the total fatty acids minus the insoluble. It is unnecessary to make the actual determination in most instances. When desired for some particular purpose, however, the test may be carried out as follows:

Reagents.--Alcohol, redistilled, free from acids and aldehydes.

Alcoholic potash solution, 50 c.c. of a saturated solution of potassium hydroxide, free from carbonate, to 1000 c.c. of alcohol. The alkali should be added to the alcohol slowly, with agitation, in order to prevent any appreciable rise in temperature. The solution should be allowed to stand at least 24 hours and filtered immediately before use.

N/2 sulfuric acid.

N/2 potassium (or sodium) hydroxide.

Phenolphthalein solution, 1 gram to 100 c.c. of alcohol, neutralized.

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[1] This may mean either hot or cold water, according to the method employed.

Method.--Five grams of fat are brought into a 300 c.c. Erlenmeyer flask together with 50 c.c. of alcoholic potash, accurately measured with a burette, and 50 c.c. of alcohol. The flask is connected with a spiral or other form of reflux condenser and the solution boiled on a water bath with occasional rotating of the contents until saponification is complete,--about 60 minutes. The condenser is then removed and the flask placed in a water bath (immersed in the water) and the alcohol evaporated at a gradually increasing temperature, care being taken to prevent spattering. The last traces of alcohol, occluded in the soap, are expelled by breaking up the dry cake or by dissolving it in water and continuing the heating. Water to a volume of 100 c.c. and 1 c.c. of N/2 sulfuric acid in excess of that required to neutralize the 50 c.c. of alcoholic potash are added and the flask, connected with a spiral condenser, heated on a water bath until the separated fatty acids and underlying liquid become clear. From this point the process is conducted the same as for insoluble acids using a spiral condenser to prevent loss of volatile acids. The combined filtrate and washings are titrated with N/2 potassium hydroxide, using phenolphthalein as indicator. The difference between the titration of the test and that of the excess N/2 acid (1 c.c.) is the alkali equivalent of the soluble acids in the fat taken which should be calculated to milligrams of potassium hydroxide for 1 gram of fat.

One c.c. of N/2 alkali is equivalent to 28.054 milligrams of potassium hydroxide.

The percentage of soluble fatty acids (S) is calculated from the number of milligrams of potassium hydroxide (k) required to neutralize the soluble acids in 1 gram of fat and the determined (or estimated) neutralization number (n) of the soluble acids by the formula:

$$S = \frac{k}{n}$$

Limit of error, 0.25 percent soluble acids.

Supplementary Notes.--The solubility of acids of the acetic series decreases with the increase in number of carbon atoms in the molecule. The so-called soluble acids include capric and all acids of less carbon atoms.

Acid.		Soluble in 100 parts of Water.	
		15° C.	100° C.
Acetic	v.s.[1]	∞	∞
Butyric	v.s.	∞	∞
Valeric	s.	3.70	-
Caproic	d.s.	.882	-
Caprylic	i.	.079	.25
Capric	i.	ins.	.10

Lauric is classes as "insoluble" although slightly soluble in boiling water.

A soluble dibasic acid occurs in Japan wax which is not volatile.

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[1] Solubility based on Mulliken's classification, Identification of Pure Organic Compounds, 1, p. 38 (1911).

	1 Gram in c.c:	Grams in 100 c.c:
Very soluble, ...	5	20
Easily soluble, ...	5-20	20-5
Soluble, ...	20-50	5-2
Difficultly soluble, . . .	50-150	2-.66
Very difficultly soluble, .	150-500	.66-.2
Insoluble, ...	500-over	.2 -

Neutralization Number.--The neutralization number indicates the milligrams of potassium hydroxide required to neutralize 1 gram of soluble fatty acids.

The difference between the saponification number (s) of the fat and the product of the percentage of insoluble fatty acids (I) times their neutralization number (n_1) indicates the milligrams of potassium hydroxide required to neutralize the soluble fatty acids in 1 gram of fat, which, divided by the percentage of soluble fatty acids (S), gives the neutralization number (n) of the soluble fatty acids.

$$n = \frac{s - In_1}{S}$$

Mean Molecular Weight.--The molecular weight (m) of the soluble fatty acids can be calculated from the neutralization number (n) by means of the formula:

$$m = \frac{56108}{n}$$

Neutralization Numbers of Soluble Fatty Acids.

Acid.	Formula.	Molecular Weight.	Neutralization Number.
Acetic, ...	CH_3COOH	60.032	934.635
Butyric, ...	C_3H_7COOH	88.064	637.128
Valeric, ...	C_4H_9COOH	102.080	549.647
Caproic, ...	$C_5H_{11}COOH$	116.096	483.290
Caprylic, ...	$C_7H_{15}COOH$	144.128	389.393
Capric, ...	$C_9H_{19}COOH$	172.160	325.906

Glycerides of Soluble Fatty Acids.--The amount of triglycerides (Sg) can be calculated from the amount (S) and molecular weight (m) of the soluble fatty acids by the formula:

$$Sg = \frac{3m + C_3H_2}{3m} \times S \quad \text{or}$$

$$\frac{3m + 38.016}{3m} \times S$$

Glycerol in the Glycerides of Soluble Acids.--The amount of glycerol (G) required for combination with the soluble acids to form triglycerides (Sg) can be calculated from the amount (S) and molecular weight (m) of the soluble acids.

$$G = \frac{C_3H_5(OH)_3}{3m + C_3H_2} \times Sg \quad \text{or}$$

$$\frac{92.064}{3m + 38.016} \times Sg$$

Substituting the value of the glycerides in terms of m and S:

$$G = \frac{92.064}{3m + 38.016} \times \frac{3m + 38.016}{3m} \times S \quad \text{or}$$

$$\frac{92.064}{3m} \times S$$

See table "Percentage of Fatty Acids and Glycerol in Triglycerides".

Mean Molecular Weight and Saponification Number of the Glycerides of the Soluble Acids.--The mean molecular weight (m_2) and saponification number (s_2) of the glycerides of the soluble acids can be calculated from the molecular weight (m) of the soluble acids.

$$m_2 = 3m + 38.016$$

$$s_2 = \frac{3 \times 56108}{m_2}$$

See table "Saponification Number of Triglycerides".

From the above formulas, the following factors were deduced for the soluble acids enumerated below. The percentage of triglycerides and of glycerol may be calculated more easily from the amount of fatty acids by means of the factors.

Acid.	Factor for Percent of Triglycerides.	Factor for Percent of Glycerol.
Acetic, ...	1.21109	.51119
Butyric, ...	1.14390	.34847
Valeric, ...	1.12414	.30063
Caproic, ...	1.10915	.26433
Caprylic, ...	1.08792	.21292
Capric, ...	1.07361	.17825

Glycerides of Soluble Fatty Acids.--The amount of triglycerides (Sg) can also be calculated from the amount (S) of soluble fatty acids and the milligrams (k) of potassium hydroxide required to neutralize the soluble acids in 1 gram of fat by the formula:--

$$C_3H_2 = \frac{38.016}{168.328} \times k \quad \text{or}$$

$$.2258\ k$$

$$Sg = \left(\frac{38.016}{168.328} \times k\right) + S \quad \text{or}$$

$$.2258\ k + S$$

REICHERT-MEISSL NUMBER.

The Reichert-Meissl number[1] indicates the cubic centimeters of N/10 potassium hydroxide required to neutralize that portion of the volatile fatty acids which is obtained from 5 grams of an oil, fat or wax by the Reichert distillation process[2].

Reagents.--Glycerol potash solution, 120 grams of potassium hydroxide free from carbonate, to 1000 c.c. of pure glycerol, heated sufficiently to dissolve the alkali (about 105° C).

Sulfuric acid, 1 to 4.

N/10 potassium (or sodium) hydroxide solution.

Phenolphthalein solution, 1 gram to 100 c.c. of alcohol, neutralized.

Glass beads, weighing approximately .5 gram each.

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[1] The Reichert-Meissl number is about 2.2 times as great as the Reichert. Lewkowitsch, Analysis of Oils, Fats and Waxes, 1, p. 417 (1913).

[2] Ztschr. Analyt. Chem. 18, pp. 68-73 (1879).

Method.--Into an Erlenmeyer flask of 300 c.c. capacity are brought 5 grams of fat (care being taken to avoid getting any fat on the sides of the flask), together with 20 c.c. of glycerol potash and heated over a small naked flame, rotating continuously, until the saponification is complete, as shown by the mixture becoming perfectly clear. Care should be taken not to overheat and discolor.the material. The soap when cold should be absolutely free from globules of fat. Twenty grams of glass beads, 135 c.c. of recently boiled distilled water and 5 c.c. of sulfuric acid (1-4) are added, and the flask connected with a Liebig condenser[1]. The mixture is heated on 20 mesh iron gauze at low ebullition until the separated fatty acids and underlying liquid become clear. One hundred and ten c.c. are then distilled as nearly as possible in 30 minutes, and received in a graduated flask. The flame should be well oxidized to induce vigorous agitation of the beads, thus assuring a more thorough separation of the volatile acids. The distillate is thoroughly mixed and passed through a dry, dense filter to remove all traces of higher fatty acids that appear as oily drops or white solid particles. One hundred c.c. are pipetted into an Erlenmeyer flask and titrated with N/10 alkali, using 1 c.c.[2] of phenolphthalein as indicator, avoiding entirely the addition of water. The pink coloration should hold several minutes. Care should be exercised at all times during the process to prevent the absorption of carbonic acid. Blank determinations should be run with every new lot of reagents. The titration reading, minus the blank, increased by one-tenth and reduced to a 5 gram fat basis is the Reichert-Meissl number.

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[1] A vertical condenser with a rapid circulation of cold water is advisable.

[2] A definite quantity is necessary if the mean molecular weight is to be determined.

Limit of error, 0.25 Reichert-Meissl number.

Synopsis of Reaction.--

$$(RCOO)_3C_3H_5 + 3KOH = 3RCOOK + C_3H_5(OH)_3$$

fat	alkali	salt	glycerol

The glycerol acts as a transmitter of heat, having a boiling point of 290° C.

$$2RCOOK + H_2SO_4 = 2RCOOH + K_2SO_4$$

Distillation of the volatile acids. Titration.

Supplementary Notes.--As this method is only an arbitrary one, it is essential to adhere strictly to the conditions of operation as laid down if comparative results are to be obtained, and by so doing, over 80 percent of the soluble acids in butter can be secured in the distillate. Jensen states that the Reichert process[1] yields with butter fat 85 to 88 percent of the total butyric, 24 to 25 percent of the caprylic, and 85 to 100 percent of the capric acids. Repeated distillation yields higher results, but is accompanied by decomposition of the nonvolatile acids. Glycerol potash is preferable to alcoholic potash in that it shortens the process and prevents possible loss due to the formation of esters during saponification. Sodium hydroxide has greater basicity than potassium hydroxide, but the resulting hard soap is less soluble. The fatty acids appear to have practically the same affinity for both hydroxides.

Acetic, butyric, valeric, caproic, caprylic and capric are the only fatty acids that can be distilled under ordinary pressure without decomposition. These acids have comparatively high boiling points, as shown by the following table, but owing to their high vapor tension they can be readily distilled from aqueous solutions with steam, and are termed "volatile" acids.

Acid.	Boiling Point. (°C.)
Acetic acid, ...	118.1
Butyric acid, ...	162.3
Valeric acid, ...	186.0 to 186.4
Caproic acid, ...	202.0 " 203.0
Caprylic acid, ...	236.0 " 237.0
Capric acid, ...	268.0 " 270.0

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[1] Ztschr. Untersuch. Nahr. u. Genussmtl., 1905, p. 272.

The boiling points rise with the increase in molecular weight. Lauric acid is very slightly volatile in a current of steam. The nonvolatile acids when distilled at ordinary pressure undergo partial decomposition and yield hydrocarbons of the ethane series, and possibly of the ethylene and aromatic series as in the case of glycerides. In vacuo they can be distilled with or without superheated steam.

Most of the natural fats and oils contain but a small amount of volatile (soluble) fatty acids, generally below 2, Reichert-Meissl number. Some prominent exceptions have already been enumerated, being characterized by saponification numbers exceeding 210. See supplementary notes under "Saponification Number". The high Reichert-Meissl numbers of dolphin and porpoise oils may be due to valeric acid. The amount of volatile or soluble acids in those oils and fats whose saponification number does not exceed 195 is inappreciable.

Among the oils and fats with a high volatile acid content might be mentioned[1] myrtle seed oil, croton oil, oleander oil, senega root oil, lycopodium oil, apeiba oil, dolphin oil, macassar oil, muriti fat, mocaya oil, palm kernel oil, cocoaut oil and tonka butter having Reichert-Meissl numbers between 5 and 15, and spindle tree oil, malukang oil, dolphin jaw oil, porpoise body oil, porpoise jaw oil, brown fish oil and butter fat having Reichert-Meissl numbers ranging from 25 to 50 or even greater in some instances.

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[1] Lewkotitsch, Analysis of Oils, Fats and Waxes, 1, p. 423 (1913).

The amount of volatile acids is likely to increase with the age of the sample.

The results of Wechsler[1] and other investigators indicate that volatile acids of higher molecular weight distill over before the lower acids, especially in cases where the neutrality of the solution is destroyed gradually by several additions of acid instead of one, thus fractionating the distillates.

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[1] Jour. Soc. Chem. Indus., 1894, p. 179. (From Lewkowitsch).

VOLATILE ACIDS.

The percentage of volatile acids (V) can be calculated from the Reichert-Meissl number and the neutralization number (n) of the volatile acids by the following formula:

$$V = \frac{0.2\ \text{R-M. No.} \times 5.6108}{n}$$

Formulas for calculating the amount of triglycerides of the volatile fatty acids, the mean molecular weight and saponification number of the triglycerides and glycerol content will be found under like headings of "Soluble Acids".

POLENSKE NUMBER.

The Polenske number indicates the c.c. of N/10 potassium hydroxide required to neutralize that portion of the insoluble volatile acids which is obtained from 5 grams of an oil, fat or wax by the Reichert distillation process.

Reagents.--Glycerol potash solution, 120 grams of potassium hydroxide, free from carbonate, to 1000 c.c. of pure glycerol, heated sufficiently to dissolve the alkali (about 105° C.).

Sulfuric acid, 1 - 4.

N/10 potassium (or sodium) hydroxide solution.

Alcohol, redistilled, free from acids and aldehydes.

Phenolphthalein solution, 1 gram to 100 c.c. of alcohol, neutralized.

Alkali blue (6B) solution, 1 gram to 100 c.c. of alcohol. The indicator should be boiled in a flask under a reflux condenser for 2 hours and then filtered.

Method.--The method can be conducted in connection with the Reichert-Meissl test and may be considered supplementary to it. A vertical condenser should be employed with a circulation of water adequate to chill the distillate to 20° C., and the test carried out as usual. The resulting distillate is chilled 15 minutes at 15° C., carefully mixed by reversing the flask several times (avoiding any shaking) and poured through a dry 11 c.m. filter. The condenser, 110 c.c. flask and filter are washed three times in succession with 15 c.c. of water to remove soluble acids, and the wash waters thrown away. The insoluble volatile acids in the condenser, 110 c.c. flask and filter are dissolved in alcohol and titrated with N/10 alkali, using 1 c.c. of phenolphthalein as indicator. The titration reading minus the blank, reduced to a 5-gram basis, is the Polenske number.

Limit of error, .10 Polenske number.

Synopsis of Reaction.--Solution of insoluble volatile acids in alcohol. Titration.

Supplementary Notes.--The Polenske number for most oils and fats having a saponification number of about 195 rarely exceeds .65 unless the product is excessively acid or rancid.[1] The Polenske number of butter fat is about 2 to 3, of palm kernel oil 10 to 12, and of coconut oil 15 to 20.

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[1] Lewkowitsch, Analysis of Oils, Fats and Waxes, I, p. 426 (1913).

INSOLUBLE FATTY ACIDS AND UNSAPONIFIABLE MATTER.

(HEHNER NUMBER)[1].

The insoluble fatty acids of an oil, fat or wax indicate (unless otherwise stated) the percentage of fatty acids and unsaponifiable matter that is insoluble in water[2].

Reagents.--Glycerol potash solution, 120 grams of potassium hydroxide, free from carbonate, to 1000 c.c. of pure glycerol, heated sufficiently to dissolve the alkali (about 105° C.).

Sulfuric acid, 1 - 4.

Ceresine, pure white, filtered.

Ethyl ether, anhydrous and free from alcohol and residue.

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[1] Angell and Hehner, Butter, Its Analysis and Adulteration, (1874).

[2] This may mean either hot or cold water according to the method employed.

Method.--Five grams of fat are brought into a 300 c.c. Erlenmeyer flask together with 20 c.c. of glycerol potash and heated over a small naked flame, rotating continuously until the saponification is complete, as shown by the mixture becoming perfectly clear. Care should be taken not to overheat and discolor the material. The resulting soap, absolutely free from globules of fat, is dissolved in 150 c.c. of hot water and decomposed with a slight (few drops) excess of sulfuric acid (1-4)[1]. The flask, loosely stoppered,[2] is heated on a water bath with occasional agitation, until the separated fatty acids and underlying liquid become clear. This requires a number of hours, generally over night, and must not be slighted. The flask is immersed in cold water[3] to solidify the fatty acids, after which the solution is decanted through a dense, ether-extracted filter[4], care being taken not to break the insoluble cake. One hundred and fifty c.c. of boiling water are added, thoroughly agitated, heated as above, cooled and decanted, the process being repeated until the washings are free from acid. Litmus paper is not sufficiently sensitive for this purpose. The final 150 c.c. of filtrate should give a decided color with 3 or 4 drops of N/10 alkali, using phenolphthalein as indicator. In those cases where caprylic and particularly capric acids are present the filtrate will often give an appreciable acid reaction after 15 to 20 washings. The treatment should be continued until the acidity of the filtrate is less than 0.25 c.c. N/10 solution.

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[1] About 5 c.c. are required.

[2] A reflux condenser is necessary if the soluble acids are to be determined.

[3] A flat-bottomed "plug" sink with the outlet closed with a perforated cork carrying a piece of glass tubing to regulate the height of the water serves quite satisfactorily as a chilling bath.

[4] Baker & Adamson Chemical Co., 12.5 c.m. washed filter paper

In determining the insoluble acids of oils and fats having a low solidifying point, the addition of .5 to 1 gram of ceresine[1] before treating with sulfuric acid greatly facilitates subsequent work and serves to protect the unsaturated acids from decomposition.

The filter and inverted flask containing the cake of insoluble fatty acids are allowed to drain in a cool place until practically dry. A convenient filter stand for both filtration and draining is illustrated by Wiley.[2] The small particles of fat adhering to the filter are dissolved in ether in a continuous fat extractor[3] run into the flask, and the ether expelled in the usual manner. The ether fumes are very persistent and necessitate blowing out the flask with hand bellows. The insoluble acids are dried in an air bath at 100° C., or in a vacuum oven at 70° C., to approximately constant weight. At 100° C. the drying periods should not exceed 2 hours. The weight of the flask is determined at the completion of the test to offset the solvent action of the reagents on the glass. Blanks should be run on every new lot of ceresine to determine the amount (if any) of soluble material present.

There are compensating errors that usually result from this method, namely, volatilization of fatty acids, dehydration of simple and hydroxy fatty acids with the formation of anhydrides and of lactones respectively, and oxidation of unsaturated acids[4]. Drying in a vacuum oven at 70° C. in a current of carbonic acid gas or even of dry air, will reduce oxidation as well as dehydration and volatilization.

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[1] The amount required varies with the consistency of the insoluble acids that are being determined.

[2] Foods and Food Adulterants, U. S. Dept. Agr., Bur. Chem., Bul. 13, p. 457.

[3] An apparatus such as is used in fodder analysis.

[4] Saturated acids do not readily absorb oxygen. Unsaturated acids of the linolic and linolenic series absorb oxygen from the air at ordinary temperatures and of the oleic series at higher

Limit of error, 0.25 percent insoluble acids.

Synopsis of Reaction.--Similar to those of Reichert-Meissl number.

Supplementary Notes.--Differences in chemical structure of the insoluble fatty acids permit of their classification into saturated, unsaturated, hydroxy acids, etc.

The principal saturated acids are lauric, myristic, palmitic, stearic, arachic and dihydroxystearic.

The most prominent unsaturated acids are oleic, erucic, linolic, linolenic, clupanodonic and ricinoleic. When these acids are compared with the empirical formula for saturated acids, $C_nH_{2n+1}COOH$, they show a deficiency of 2, 4, 6, or 8 atoms of hydrogen, which indicates their power to absorb iodine chloride with the formation of additive compounds. Members of the chaulmoogric and tariric series constitute an exception to the above statement. (See "Calculated Data from the Iodine Number".). Unsaturated acids containing an open chain of 18 carbon atoms (oleic, linolic, linolenic acids, etc.), are reduced by hydrogen in the presence of a suitable catalyzer (nickel or colloidal palladium) to stearic acid.

Dihydroxystearic and ricinoleic acids are hydroxy acids which on acetylation assimilate an acetyl radical (CH_3CO) in place of the hydrogen in every alcoholic hydroxyl group.

Most fats and oils contain from 93 to 96 percent of insoluble acids, with a mean of approximately 95. Some notable exceptions, having saponification numbers exceeding 210 and a high volatile acid content, have already been mentioned. The fatty acids are practically all insoluble where the saponification number of an oil or fat does not exceed 195. Croton oil contains about 89 percent of insoluble acids and unsaponifiable matter, dolphin jaw oil 66 percent, porpoise jaw oil 70 percent, brown fish oil 85.5 percent, laurel oil 83.5 to 87 percent, palm nut oil 87.5 to 91 percent, coconut oil 88 to 90 percent, Japan wax 90.5 percent and butter 86.5 to 90 percent.

In liquid waxes the amount of insoluble fatty acids free from alcohols and hydrocarbons varies from 60 to 65 percent, and in solid waxes from 47 to 60 percent.

The acid content of palmitin is 95.29, stearin 95.73, olein 95.70, linolein 95.67, and linolenin 95.64 percent; therefore, the percentage of insoluble acids in most oils and fats free from appreciable amounts of the lower fatty acids and of unsaponifiable matter must be in the vicinity of 95.

PREPARATION OF INSOLUBLE ACIDS.

The method for preparing insoluble fatty acids for analysis is the same as described for the determination of "Insoluble Fatty Acids and Unsaponifiable Matter", with the elimination of such features as are necessary only for quantitative work. Eleven c.c. (10 grams) of the melted fat or oil are pipetted into a 300 c.c. Erlenmeyer flask together with 40 c.c. of glycerol potash and saponified. The resulting soap is dissolved in 150 c.c. of hot water and decomposed with a slight excess (few drops) of sulfuric acid (1-4), heating on a water bath, with occasional agitation, until the separated fatty acids and underlying liquid become clear. Several such charges will furnish sufficient material for the analysis. The fatty acids may be washed as described under "Insoluble Fatty Acids and Unsaponifiable Matter", or the contents of the flasks transferred to a separatory funnel and washed, by shaking out with hot water[1], until free from soluble acids. The latter modification has some advantages particularly for insoluble acids of low melting point. Thorough washing may not always insure the entire removal of caprylic and capric acids when present, but the treatment should not be unduly prolonged from fear of injury to the unsaturated acids. The melted fatty acids are run into a test tube, heated in a water bath at 60° C. to allow any water present to settle out, filtered in a jacketed funnel and preserved in a tightly stoppered bottle in a cool, dark place.

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[1] The layer of fatty acids should be allowed to partially solidify at least before the water is drawn off to insure conditions similar to those prevailing in the quantitative determination of the insoluble acids.

The above process should yield fatty acids practically free from decomposition. It is inadvisable to employ the residue from the quantitative determination of the insoluble acids for further tests.

Neutralization Number.--The neutralization number indicates the milligrams of potassium hydroxide required for the complete neutralization of 1 gram of insoluble fatty acids, by the saponification process.

Reagents.--Alcohol, redistilled, free from acids and aldehydes.

Alcoholic potash solution, 50 c.c. of a saturated solution of potassium hydroxide, free from carbonate, to 1000 c.c. of alcohol. The solution should be allowed to stand at least 24 hours and filtered immediately before use.

N/2 hydrochloric acid.

Phenolphthalein solution, 1 gram to 100 c.c. of alcohol, neutralized.

Alkali blue (6B) solution, 1 gram to 100 c.c. of alcohol. The indicator should be boiled in a flask under a reflux condenser for 2 hours and then filtered.

Method.--Into a 300 c.c. Erlenmeyer flask are brought 5 grams of insoluble fatty acids, together with 50 c.c. of alcoholic potash accurately measured with a burette, 50 c.c. of alcohol and several glass beads. The flask is then connected with a spiral or other form of reflux condenser and the solution boiled on a water bath, with occasional rotating of the contents, until the reaction is complete, about 60 minutes. The flask is then placed in a water bath at 60° C. and the solution, after cooling to that temperature, titrated with N/2 hydrochloric acid, using 1 c.c. of phenolphthalein or alkali blue as indicator. For further details see "Saponification (Koettstorfer) Number". The difference between the titration of the blank and that of the excess alkali of the test is the acid equivalent of the insoluble acids taken which should be calculated to milligrams of potassium hydroxide for 1 gram of insoluble acids.

One c.c. of N/2 acid is equivalent to 28.054 milligrams of potassium hydroxide.

Limit of error, 0.50 neutralization number.

Synopsis of Reaction.--See "Acid Number" with titration of excess alkali as in "Saponification Number".

Mean Molecular Weight.--The molecular weight (m) of the insoluble fatty acids can be calculated from the neutralization number (n) by means of the formula:

$$m = \frac{56108}{n}$$

or directly from the acid equivalent:

$$m = \frac{2000 \times \text{wt. of fatty acids}}{\text{c.c. N/2 acid}}$$

Neutralization Number and Mean Molecular Weight.--The neutralization number (n) and mean molecular weight (m) of the insoluble fatty acids can be calculated from the amount (I) of acids and the milligrams (k) of potassium hydroxide required to neutralize the acids in 1 gram of fat:

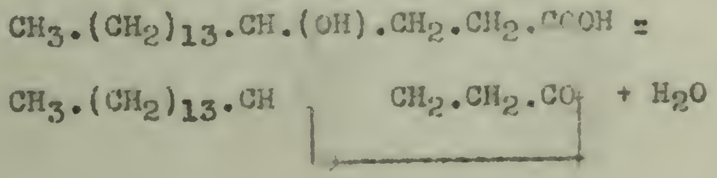
$$n = \frac{k}{I}$$

$$m = \frac{56108}{n} \quad \text{or}$$

$$\frac{56108 \times I}{k}$$

These formulas are exactly the same as those previously stated except the data are expressed differently.

Supplementary Notes.--Direct titration of the fatty acids in alcohol with N/2 alkali in a manner similar to that of "Acid Number" is often recommended although the process tends to yield low neutralization numbers[1], probably due to the presence of lactones (inner anhydrides) of hydroxy acids or of anhydrides of fatty acids which do not combine with aqueous alkali in the cold, but readily hydrolyze on boiling with alcoholic potash. The lactones and anhydrides may result entirely from drying under unfavorable conditions. Marked differences between the two methods are shown in the references cited. In our hands, working with purified insoluble fatty acids, only slight differences were obtained except in the case of oleic acid. The saturated acids were prepared with special precautions and dried at a low temperature which precluded the possibility of forming anhydrides. Hydroxy acids such as ricinoleic acid of castor oil are particularly likely to dehydrate with the formation of inner anhydrides. Lactones occur naturally or are readily formed from the fatty acids of Sewarri fat and of wool wax. In the latter case they result to some extent from heating at 100° C. The fatty acids of castor oil polymerize on long standing even at ordinary temperature to polyricinoleic acid. Gamma dihydroxystearic acid on losing a molecule of water forms stearolactone and may serve as an example (Lewkowitsch):



$$CH_3.(CH_2)_{13}.CH.(OH).CH_2.CH_2.COOH =$$

$$CH_3.(CH_2)_{13}.CH \quad CH_2.CH_2.CO + H_2O$$

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[1] Tortelli and Pergami, L'Orosi, 1901, p. 1. (From Lewbowitsch)
Lewkowitsch, Analysis of Oils, Fats and Waxes, I, pp. 518-519 (1913).

Neutralization Number of Insoluble Fatty Acids.

Acid.	Formula.	Molecular Weight.	Neutralization Number.
Lauric, ...	$C_{11}H_{23}COOH$	200.192	280.271
Myristic, ...	$C_{13}H_{27}COOH$	228.224	245.846
Palmitic, ...	$C_{15}H_{31}COOH$	256.256	218.953
Stearic, ...	$C_{17}H_{35}COOH$	284.288	197.363
Arachic, ...	$C_{19}H_{39}COOH$	312.320	179.649
Oleic, ...	$C_{17}H_{33}COOH$	282.272	198.773
Erucic, ...	$C_{21}H_{41}COOH$	338.336	165.835
Linolic, ...	$C_{17}H_{31}COOH$	280.256	200.203
Linolenic, ...	$C_{17}H_{29}COOH$	278.240	201.653
Clupanodonic, ...	$C_{17}H_{27}COOH$	276.224	203.125
Ricinoleic, ...	$C_{17}H_{32}.OH.COOH$	298.272	188.110
Dihydroxystearic, . . .	$C_{17}H_{33}(OH)_2COOH$	316.288	177.395

Glycerides of Insoluble Fatty Acids.--The amount of triglycerides (Ig) can be calculated from the amount (I) and molecular weight (m) of the insoluble fatty acids by the formula:

$$Ig = \frac{3m + C_3H_2}{3m} \times I \quad \text{or}$$

$$\frac{3m + 38.016}{3m} \times I$$

Glycerol in the Glycerides of Insoluble Acids.--The amount of glycerol (G) required for combination with the insoluble acids to form triglycerides (Ig) can be calculated from the amount (I) and molecular weight (m) of the insoluble acids:

$$G = \frac{C_3H_5(OH)_3}{3m + C_3H_2} \times Ig \quad \text{or}$$

$$\frac{92.064}{3m + 38,016} \times Ig$$

Substituting the value of the glycerides in terms of m and I:

$$G = \frac{92.064}{3m + 38.016} \times \frac{3m + 38.016}{3m} \times I \quad \text{or}$$

$$\frac{92.064}{3m} \times I$$

See table "Percentage of Fatty Acids and Glycerol in Triglycerides".

Mean Molecular Weight and Saponification Number of the Glycerides of the Insoluble Acids.--The mean molecular weight (m_1) and saponification number (s_1) of the glycerides of the insoluble acids can be calculated from the molecular weight (m) of the insoluble acids:

$$m_1 = 3m + 38.016$$

$$s_1 = \frac{3 \times 56108}{m_1}$$

See table "Saponification Number of Triglycerides".

From the above formulas the following factors were deduced for the insoluble acids enumerated below. The percentage of triglycerides and of glycerol may be calculated more easily from the amount of fatty acids by means of these factors:

Acid.	Factor for Percent of Triglycerides.	Factor for Percent of Glycerol.
Lauric, ...	1.06330	.15329
Myristic, ...	1.05552	.13446
Palmitic, ...	1.04945	.11976
Stearic, ...	1.04457	.10795
Arachic, ...	1.04057	.09826
Oleic, ...	1.04489	.10872
Erucic, ...	1.03745	.09070
Linolic, ...	1.04522	.10950
Linolenic, ...	1.04554	.11029
Clupanodonic, ...	1.04588	.11110
Ricinoleic, ...	1.04248	.10289
Dihydroxystearic, ...	1.04006	.09703

Glycerides of Insoluble Fatty Acids.--The amount of triglycerides (Ig) can also be calculated from the amount (I) of insoluble fatty acids and the milligrams (k) of potassium hydroxide required to neutralize the insoluble acids in 1 gram of fat by the formula:

$$C_3H_2 = \frac{38.016}{168.328} \times k \quad \text{or}$$

$$.2258\ k$$

$$Ig = \left(\frac{38.016}{168.328} \times k\right) + I \quad \text{or}$$

$$.2258\ k + I$$

Lactones and Anhydrides.--The amount of lactones and anhydrides present in the separated insoluble acids of oil, fats and waxes can be measured in terms of milligrams of potassium hydroxide, by the difference in the amount of alkali required to titer (neutralize) the acids in cold alcohol and that absorbed on saponifying with alcoholic potash. Lactones and anhydrides are unable to combine with alkali until transformed into acids. They are not hydrolyzed to any considerable extent in cold alcohol but are readily hydrolyzed by boiling alcoholic potash.

Reagents.--Same as for "Acid" and "Saponification" Numbers.

Method.--Five grams of insoluble fatty acids are brought into a 300 c.c. Erlenmeyer flask together with 100 c.c. of alcohol and titrated in the cold with N/2 potassium hydroxide, using 1 c.c. of phenolphthalein ar alkali blue as indicator. An additional 5 grams of insoluble acids are brought into a flask and treated exactly as described for Saponification Number. The difference between the two determinations, in terms of milligrams of potassium hydroxide to the gram of insoluble acids, measures the amount of lactones and anhydrides present.

Limit of error, same as in the determinations of Acid Number and Saponification Number.

Synopsis of Reaction.--Solution in alcohol.

Neutralization:

$$\underset{\text{acid}}{RCOOH} + \underset{\text{lac-tone}}{ROCO} + \underset{\text{anhy-dride}}{(RCO)_2O} + KOH = RCOOK + ROCO + (RCO)_2O + H_2O$$

Saponification:

$$RCOOH + ROCO + (RCO)_2O + 4KOH = RCOOK + R.OH.COOK + 2RCOOK + 2H_2O$$

When the lactone or anhydride in the insoluble acids or the predominant one is a mixture is known, the amount (L) can be calculated from the determined alkali equivalent[1] (l); i.e., the saponification number minus the acid number of insoluble acids and the theoretical saponification number (s_x) of the lactone or anhydride by the formula:

$$L = \frac{l}{s_x}$$

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[1] Lactone Number.

Iodine Number.--The iodine number indicates the percentage of iodine chloride absorbed by the insoluble fatty acids, expressed in terms of iodine.

See method for oils and fats.

The iodine number of insoluble acids does not necessarily correspond to that of the natural oil, fat or wax from which the acids were derived. This is said to be due to the influence of soluble fatty acids in the natural product, although it is probable that some decomposition of the unsaturated acids results in the process of separation.

Insoluble acids after titration with thiosulfate undergo a reversible reaction and split off iodine much more rapidly than the oils and fats from which they were derived.

Acetyl Number.--The acetyl number indicates the milligrams of potassium hydroxide required to combine with the acetyl absorbed by 1 gram of insoluble fatty acids on acetylation.

See method for oils and fats.

The acetylated product should be washed until the acidity of the filtrate (150 c.c.) is less than 0.25 c.c. N/10 solution.

IODINE NUMBER.

The iodine number indicates the percentage of iodine chloride absorbed by an oil, fat or wax, expressed in terms of iodine.

Hubl Method. Wijs Solution.

Reagents.--Iodine solution according to Wijs[1]. Thirteen grams of resublimated iodine to 1000 c.c. of anhydrous acetic acid[2] (99.9 percent), free from oxidizable products. After the iodine is completely dissolved the solution is treated with pure dry chlorine gas[3] until the iodine has been converted into monochloride. The completion of the reaction is indicated by a distinct change, the solution becoming transparent cherry red, and its titer[4] with thiosulfate doubled. As it is advisable to have a slight excess of iodine a small quantity of untreated solution should be retained and may be added in case of necessity.

N/10 sodium thiosulfate (hyposulfite) solution, 24.822 grams[5] of sodium thiosulfate are dissolved in water and made up to a liter.

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[1] Ber. Deut. Chem. Gesell. 31, p. 750 (1898). Wijs' solution, with the same active reagent, iodine monochloride, has largely replaced that of Hubl on account of its far greater stability and more rapid absorption.

[2] The acid should be crystallized at 15° C. and the mother liquor discarded. The acid should not react with the bichromate test.

[3] Washed and dried by being passed through concentrated sulfuric acid. Gas sufficient for 4000 c.c. of iodine solution can be generated from 44.5 grams of sodium chloride, 55.5 grams of manganese dioxide and 150 c.c. of sulfuric acid (1-1).

[4] With the addition of potassium iodide as usual.

[5] Preferably 50 grams to 1000 c.c. of water.

Potassium bichromate solution; 3.8633 grams of dry C. P. potassium bichromate, free from sodium bichromate, are dissolved in water and made up to a volume of 1000 c.c. at 20° C. This solution will keep almost indefinitely without changing and is used for standardizing the thiosulfate solution. One hundred c.c. of potassium bichromate will liberate 1 gram of iodine from a potassium iodide solution.

Potassium iodide solution; 165 grams of neutral potassium iodide, free from iodine and iodate, to 1000 c.c. of water. Iodate is said to be present frequently in commercial potassium iodide and yields free iodine with hydrochloric acid.

Starch paste; 1 gram to 200 c.c. of water. The indicator is prepared by boiling thoroughly, decanting and diluting the solution, and again boiling to insure a perfect paste free from solid particles.

Carbon tetrachloride, anhydrous[1] and free from oxidizable products.[2]

Standardizing the Thiosulfate.--Twenty-five c.c. of potassium bichromate are accurately measured with a burette into a 300 c.c. Erlenmeyer flask and 10 c.c. of potassium iodide and 5 c.c. of concentrated hydrochloric acid added. Simultaneous with the addition of the acid, thiosulfate is run in until the brownish yellow color (iodine) has been largely destroyed, then 2 c.c. of starch paste are added and the titration continued with repeated thorough shaking, until the blue color has entirely disappeared leaving a bright green solution. As 4 times the titration is equivalent to 1 gram of iodine, the iodine value of 1 c.c. of thiosulfate can be readily calculated.

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[1] Dried over recently ignited sodium sulfate and distilled.
[2] Bichromate test.

In theory, 1 c.c. N/10 $Na_2S_2O_35H_2O$ is equivalent to .012692 grams of iodine.

The following is the reaction:

$$K_2Cr_2O_7 + 14HCl + 6KI = 2CrCl_3 + 8KCl + 6I + 7H_2O$$

$6I : K_2Cr_2O_7 :: 1 : x$

$761.52 : 294.20 :: 1 : 0.38633$ grams in 100 c.c.

Method.--The amount of material to be taken for this determination varies inversely with its iodine number (see table). From .30 to .45 gram of drying or fish oil, .45 to .70 gram of a semidrying oil, .55 to .90 gram of a nondrying oil, or .60 to 2.00 grams of fat are brought into a 300 c.c. Erlenmeyer flask together with 20 c.c. of carbon tetrachloride. After complete solution, 50 c.c. of iodine solution, accurately measured with a burette, are added and the flask well stoppered and allowed to stand 3 to 4 hours[1], with occasional shaking, in a refrigerator at a temperature below 10° C. A rapid bleaching of the solution indicates insufficient iodine. An excess equal to the amount absorbed is deemed necessary for the attainment of constant results. The cork stopper for the flask should be rolled until soft and pliable and moistened with potassium iodide to prevent loss of iodine by volatilization. At the end of the absorption period, 50 c.c. of distilled water and 10 c.c. of potassium iodide are added to the contents of the flask, and the excess iodine titrated with sodium thiosulfate. The thiosulfate is run in gradually, with constant shaking, until the brownish yellow color of the solution has been largely destroyed, then 2 c.c. of starch paste are added and the titration continued until the blue color has entirely disappeared. Towards the end of the reaction the flask should be stoppered and shaken vigorously, so that any iodine in the carbon tetrachloride will be taken up by the potassium iodide. The "bleached" condition should hold for a considerable time with the flask stoppered, although the blue color will develop again, due to the splitting off of iodine. Several blank determinations should be run with every series of tests. The difference between the titration of the blank and that of the excess iodine is the thiosulfate equivalent of the fat, which multiplied by the factor (obtained as described) and divided by the weight of fat taken gives the percentage of iodine absorbed.

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[1] According to Lewkowitsch, 1/2 hour is sufficient for all oils and fats having an iodine number below 100, 1 hour for semidrying oils and 2 to 6 hours for drying oils. Analysis of Oils, Fats and Waxes, I, p. 407 (1913).

Limit of error, 0.25 iodine number.

Synopsis of Reaction.--Solution with carbon tetrachloride.

Formation of chloro-iodo additive compounds with unsaturated acids and their glycerides.

Solution of excess iodine with potassium iodide and titration with thiosulfate, using starch paste as indicator.

$$2\,1 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI$$

AMOUNT OF MATERIAL FOR DIFFERENT IODINE NUMBERS.

(50 c.c. of Wijs Solution).

Iodine Number.	Grams of Material.	
200	.32	
195	.33	
190	.34	
185	.34	
180	.35	
175	.36	
170	.38	
165	.39	
160	.40	
155	.41	
150	.43	
145	.44	
140	.46	
135	.47	
130	.49	
125	.51	
120	.53	
115	.55	
110	.58	
105	.61	
100	.64	
95	.67	
90	.71	
85	.75	
80	.79	
75	.85	
70	.91	
65	.98	
60	1.06	
55	1.16	
50	1.28	
45	1.42	
40	1.59	
35	1.82	
30	2.13	(Take 2 grams)
25	2.55	(" " ")
20	3.19	(" " ")

(25 c.c. of Wijs Solution).

Iodine Number.	Grams of Material.	
15	4.25	(Take 2 grams)
10	6.38	(" " ")
5	12.75	(" " ")

Supplementary Notes.--Unsaturated acids and their glycerides assimilate halogens with the formation of saturated compounds and this property serves as a basis for their quantitative determination. Theoretically chlorine, bromine, iodine, iodobromide or iodochloride may be employed for the purpose. The use of chlorine, however, is impractical and bromine tends to form both substitution and addition products. Iodobromide (Hanus solution) has no advantage over iodochloride (Wijs solution) except ease of preparation; therefore the latter process, employing the same active agent (iodine monochloride) and agreeing closely with the original Hubl method under control conditions[1], should be given preference notwithstanding American practice to the contrary. Furthermore, Wijs has shown[2] that his solution yields practically theoretical results with pure fatty acids. The solution is far more stable than that of Hubl and more rapid in its action.

Linolic and linolenic acids and their glycerides absorb oxygen from the air at ordinary temperature and dry to a hard elastic layer and to this property drying oils owe their value. It is stated that for practical purposes drying oils should have an iodine number of at least 140, preferably 170 or higher, and nondrying oils of 90 or lower. Certain fish oils have a high iodine number and will absorb oxygen but they do not dry to a hard layer.

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[1] Lewkowitsch, Analyst, 24, p. 259 (1899).
[2] Chem. Rev. Fett u. Harz Indus. 1899, p. 1.

CALCULATED DATA FROM THE IODINE NUMBER.

Theoretically the unsaturated fatty acids belonging to the oleic, chaulmoogric and ricinoleic series absorb 2 atoms of the halogen; linolic and tariric series, 4 atoms; linolenic series, 6 atoms; clupanodonic series, 8 atoms, etc. The members of the chaulmoogric series are cyclic compounds and contain only 1 pair of double-linked carbon atoms, while open chain acids of the same empirical formula would in most instances contain 2 pairs. Tariric acid contains triple-bond carbon atoms and although it forms a tetrabromide, it generally absorbs only 1 or possibly 2 halogen atoms. The double-linked carbon atoms may be considered the ethylene type and the triple-bonded an acetylene linkage. The position of the double bond in relation to the carboxyl group in unsaturated acids influences the iodine absorption. If the double bond is located at a considerable distance from the carboxyl the results are generally normal, but when relatively close together the iodine number is likely to be below theory, although lengthening the absorption period increases the results.[1] The glycerides act similarly to the free acids and absorb three times as many atoms (triglycerides). Olein is the principal unsaturated glyceride in nondrying oils and fats, linolin constitutes a considerable proportion of drying and semi-drying oils and to some extent of nondrying oils and solid fats, and linolenin occurs in large amounts in all vegetable drying oils. Clupanodonin appears to be thecharacteristic constituent of fish, liver and blubber oils.

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[1] Lewkowitsch, Analysis of Oils, Fats and Waxes, 1, p. 400 (1913).

In those cases where only one such acid or glyceride is present its percentage can be readily calculated from the iodine number by dividing by the theoretical absorption or by means of a factor.

$$\text{Oleic acid} = \frac{2\,\text{I}}{C_{17}H_{33}COOH}$$

$$= \frac{2 \times 126.92}{282.272}$$

$$= 0.89927$$

$$\text{Olein} = \frac{6\,\text{I}}{(C_{17}H_{33}COO)_3C_3H_5{}^1}$$

$$= \frac{761.52}{884.832}$$

$$= 0.86064$$

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[1] $3(C_{17}H_{33}COOH)$ + C_3H_2 or 38.016

In a similar manner the following figures for theoretical absorption were deduced for the acids and glycerides enumerated below.

	Acid.			Triglyceride.	
	Molecular Weight of Acid.	Theoretical Iodine Absorption.	Reciprocal.	Theoretical Iodine Absorption.	Reciprocal.
Oleic, . . .	282.272	0.89927	1.11201	0.86064	1.16193
Erucic, . .	338.336	0.75026	1.33287	0.72308	1.38297
Linolic, . .	280.256	1.81149	0.55203	1.73312	0.57699
Linolenic, .	278.240	2.73692	0.36537	2.61770	0.38201
Clupanodonic,	276.224	3.67586	0.27205	3.51462	0.28453
Ricinoleic, .	298.272	0.85104	1.17503	0.81635	1.22496
Sitosterol, .	386.368	0.65699	1.52209	--	--
Cholesterol,	386.368	0.65699	1.52209	--	--

Wijs solution is said to yield high and variable results with cholesterol and low results with rosin and rosin oils increasing with the excess of iodine and the length of the absorption periods.

Where there are two unsaturated acids (or glycerides) present (x and y) of known iodine absorption (c and d), if the percentage of the mixture (P) and the iodine number (W) of the fat have been determined, the percent of each acid (or glyceride) can be calculated by formula.

$$x + y = P$$

$$cx + dy = .01\ W^1$$

$$x = \frac{.01\ W - dP}{c - d}$$

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[1] The factor .01 converts the iodine number to the same basis as the figures for theoretical absorption stated on previous page.

ACETYL NUMBER.

The acetyl number indicates the milligrams of potassium hydroxide required to combine with the acetyl absorbed by 1 gram of an oil, fat or wax on acetylation.[1]

Reagents.--Acetic anhydride, Kahlbaum's.

Ceresine, pure white, filtered.

Alcohol, redistilled, free from acids and aldehydes.

Alcoholic potash, 50 c.c. of a saturated solution of potassium hydroxide, free from carbonate, to 1000 c.c. of alcohol. The solution should be allowed to stand at least 24 hours and filtered immediately before use.

N/2 hydrochloric acid.

Phenolphthalein solution, 1 gram to 100 c.c. of alcohol, neutralized.

Alkali blue (6B), 1 gram to 100 c.c. of alcohol. The indicator should be boiled in a flask under a reflux condenser for 2 hours and then filtered.

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[1] Benedikt and Ulzer, and Lewkowitsch report on the basis of the acetylated product.

Method.--Into a 300 c.c. Erlenmeyer flask are brought 5 grams of fat together with 10 c.c. of acetic anhydride. The flask is connected with a spiral or other form of reflux condenser and heated in a boiling water bath (immersed in the water) for from 1 to 1 1/2 hours. Longer heating yields higher results but is accompanied by partial decomposition of the fat with formation of aldehydes or other bodies that give a reddish color with caustic alkali. After acetylating, the spiral is removed from the flask and sufficient ceresine added to form a solid disc with the fat when chilled in cold water. The amount of ceresine required will vary with the consistency of the product under examination. For butter fat .5 gram is ample, for softer fats and oils rather more, and for harder fats, less. With the flask still in the water bath, 150 c.c. of boiling water are added with as little disturbance of the fat layer as possible. The flask is then removed and the contents rotated vigorously to dissolve occluded acetic acid. The flask is immersed in cold water to solidify the ceresine-fat, after which the solution is decanted through a dense, 12.5 c.m. filter, care being taken not to break the insoluble cake. Another 150 c.c. of boiling water is added, thoroughly agitated, heated a few minutes on the bath, cooled and decanted, the process being repeated until the final filtrate gives a decided color with 2 or 3 drops of N/10 alkali, using phenolphthalein as indicator (about six times). Prolonged washing is likely to cause some hydrolysis of the acetylated product.

The filter and inverted flask containing the cake of ceresine-fat are allowed to drain in a cool place until practically dry. The small particles adhering to the filter are then scraped into the flask, the filter washed with 5 successive 10 c.c. portions of alcohol and 50 c.c. of alcoholic potash, accurately measured with a burette, and several glass beads added. The flask is connected with a spiral or other form of reflux condenser and the solution boiled on a water bath until saponification is complete--about 60 minutes. The flask is placed in a water bath at 60° C. and the solution, after cooling to that temperature, titrated with N/2 hydrochloric acid, using 1 c.c. of phenolphthalein or alkali blue as indicator. Alkali blue offers certain advantages in the case of solutions that develop a reddish color with caustic alkali. The alcoholic mixture is again brought to boil to free any alkali occluded in the ceresine and retitered if necessary. Several blanks determinations should be run with every series of tests under precisely similar conditions as to time and treatment except that the ceresine may be omitted. However, every lot of ceresine must be tested. It should be free from soluble matter and not assimilate any alkali on saponification. The difference between the titration of the blank and that of the excess alkali in the test is the acid equivalent of the fat after acetylation, which should be calculated to milligrams of potassium hydroxide for 1 gram of fat.

One c.c. of N/2 acid is equivalent to 28.054 milligrams of potassium hydroxide.

The difference between the saponification number of the fat before and after acetylation is the acetyl number. In case the original fat contains free soluble acids their titer should be determined and proper correction made for the same.

Limit of error, 0.50 acetyl number.

Synopsis of Reaction.--Acetylation of glycerides of monohydroxy and dihydroxy acids, monoglycerides and diglycerides and free alcohols. (See formulas).

Saponification of the acetylated product. (See formulas).

Saponification of the original or unacetylated product.

Titration of excess alkali.

Acetyl number by difference.

Glycerides of Monohydroxy and Dihydroxy Acids.

Acetylation.

$$(R.OH.COO)_3C_3H_5 + 3(CH_3CO)_2O = (R.OCH_3CO.COO)_3C_3H_5 + 3CH_3COOH$$

triglyceride of monohydroxy acid	acetic anhydride	acetylated glyceride	acetic acid

Example, Ricinolein $(C_{17}H_{32}.OH.COO)_3C_3H_5$

Saponification.

$$(R.OCH_3CO.COO)_3C_3H_5 + 6KOH = 3R.OH.COOK + 3CH_3COOK + C_3H_5(OH)_3$$

acetylated glyceride	alkali	potassium salt of hydroxy acid	potassium acetate	glycerol

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$$(R(OH)_2COO)_3C_3H_5 + (CH_3CO)_2O = (R(OCH_3CO)_2COO)_3C_3H_5 + H_2O$$

triglyceride of dihydroxy acid	acetylated glyceride

Example, Dihydroxystearin $(C_{17}H_{33}(OH)_2COO)_3C_3H_5$

$$(R(OCH_3CO)_2COO)_3C_3H_5 + 9KOH = 3R(OH)_2COOK + 6CH_3COOK + C_3H_5(OH)_3$$

Monoglycerides and Diglycerides.

$(RCOO)C_3H_5(OH)_2 + (CH_3CO)_2O = (RCOO)(CH_3COO)_2C_3H_5 + H_2O$

monoglyceride diaceto-glyceride

$(RCOO)(CH_3COO)_2C_3H_5 + 3KOH = RCOOK + 2CH_3COOK + C_3H_5(OH)_3$

$(RCOO)_2C_3H_5(OH) + (CH_3CO)_2O = (RCOO)_2(CH_3COO)C_3H_5 + CH_3COOH$

diglyceride monaceto-glyceride

$(RCOO)_2(CH_3COO)C_3H_5 + 3KOH = 2RCOOK + CH_3COOK + C_3H_5(OH)_3$

Free Alcohols.

$ROH + (CH_3CO)_2O = CH_3COOR + CH_3COOH$

monobasic alcohol acetate of alcohol

$CH_3COOR + KOH = ROH + CH_3COOK$

Examples, Sitosterol, cholesterol, $C_{27}H_{45}OH$

Considerable variation is possible in writing the above formulas which, at best, poorly express the structure. In some instances the reaction is indicated at some sacrifice of form.

CALCULATED DATA FROM THE ACETYL NUMBER.

The acetyl number (c) serves to measure the amount of hydroxy compounds in an oil, fat or wax and in case only one such compound of known molecular weight (m) and number of hydroxyls (d) is present, its amount (H) can be readily calculated by the following formula:

$$H = \frac{cm}{56108d}$$

The derivation of the formula is comparatively simple. The theoretical acetyl number of a compound containing d hydroxyl groups is:

$$\frac{56108d}{m}$$

The amount of such a compound in an oil, fat or wax is, therefore,:

$$\frac{c}{\frac{56108d}{m}} = \frac{cm}{56108d}$$

The same results may be calculated more easily from the following table, dividing the determined acetyl number by the theoretical acetyl number or multiplying by its reciprocal.

Acetyl Number on Original Product.

Name.	Formula.	Molecular Weight.	Saponification Number.	Theoretical Acetyl Number.	Reciprocal.
Glycerides.					
Ricinolein	$(C_{17}H_{32}.OH.COO)_3C_3H_5$	932.832	180.444	180.444	.0055419
Dihydroxystearin	$(C_{17}H_{33}(OH)_2COO)_3C_3H_5$	986.880	170.562	341.124	.0029315
Monoglycerides.					
Monopalmitin	$(C_{15}H_{31}COO)\ C_3H_5(OH)_2$	330.304	169.868	339.736	.0029435
Monostearin	$(C_{17}H_{35}COO)\ C_3H_5(OH)_2$	358.336	156.579	313.159	.0031933
Monolein	$(C_{17}H_{33}COO)\ C_3H_5(OH)_2$	356.320	157.465	314.930	.0031753
Diglycerides.					
Dipalmitin	$(C_{15}H_{31}COO)_2C_3H_5(OH)$	568.544	197.374	98.687	.0101330
Distearin	$(C_{17}H_{35}COO)_2C_3H_5(OH)$	624.608	179.658	89.829	.0111323
Diolein	$(C_{17}H_{33}COO)_2C_3H_5(OH)$	620.576	180.826	90.413	.0110604
Hydroxy Acids.					
Ricinoleic	$C_{17}H_{32}.OH.COOH$	298.272	188.110	188.110	.0053160
Dihydroxystearic	$C_{17}H_{33}(OH)_2COOH$	316.288	177.395	354.791	.0028186
Free Alcohols.					
Sitosterol	$C_{27}H_{45}OH$	386.368	-	145.219	.0068862
Cholesterol	$C_{27}H_{45}OH$	386.368	-	145.219	.0068862

Gravimetric Process[1].--After acetylating, a gravimetric process for acetyl number may be conducted in a manner similar to that for the quantitative determination of insoluble fatty acids, observing all the precautions therein noted as to ceresine, washing, drying, weighing, etc.

This modification is apparently rather more difficult, tedious, and subject to error than the saponification or volumetric process. An inaccuracy, due to a deficiency in weight arising from the dehydration of free fatty acids by acetic anhydride during acetylation, is probably unavoidable although of little consequente where the amount of free acids is relatively small.

The acetyl number (a) is calculated from the increase in weight (i) by the following formula:

$$a = \frac{56108\ i}{42.016} \text{ or}$$

$$1335.39604 i$$

In case only one hydroxy compound of known molecular weight (m) and number of hydroxyls (d) is present, its amount can be calculated from the increase in weight (i) of the oil, fat or wax on acetylating. The theoretical increase for a hydroxy compound is:

$$\frac{42.016\ d}{m}$$

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[1] Has not received sufficient study in this laboratory to warrant positive statements but is similar to the methods described by Lewkowitsch (loco citato) 1, pp. 451-453, 578-580 (1913).

The amount (H) of such a compound in an oil, fat or wax is, therefore:

$$H = \frac{i}{\frac{42.016\ d}{m}} \quad \text{or}$$

$$\frac{im}{42.016\ d}$$

Molecular Weight of Hydroxy Compounds.--The molecular weight of the hydroxy compounds can be calculated from the weight (w) of fat taken and the increase (i) on acetylating, provided the number (d) of hydroxyls in the molecule is known:

$$w : w + i :: m : m + 42.016\ d$$

$$m = \frac{42.016\ dw}{i}$$

The formation of anhydrides during the acetylating process will affect the accuracy of these calculations.

The computation of the amount of hydroxy compounds by the gravimetric process is greatly facilitated by use of the following table.

Acetyl Gravimetric Process on Original Product.

Name.	Molecular Weight.	Molecular Weight after Acetylating.	Theoretical Increase in Weight per Gram on Acetylating.[1]	Reciprocal.
Glycerides.				
Ricinolein	932.832	1058.880	.135124	7.40061
Dihydroxystearin	986.880	1238.976	.255447	3.91471
Monoglycerides.				
Monopalmitin	330.304	414.336	.254408	3.93069
Monostearin	358.336	442.368	.234506	4.26428
Monolein	356.320	440.352	.235833	4.24029
Diglycerides.				
Dipalmitin	568.544	610.560	.073901	13.53162
Distearin	624.608	666.624	.067268	14.86591
Diolein	620.576	662.592	.067705	14.76996
Hydroxy Acids.				
Ricinoleic	298.272	340.288	.140865	7.09900
Dihydroxystearic	316.288	400.320	.265682	3.76390
Free Alcohols.				
Sitosterol	386.368	428.384	.108746	9.19574
Cholesterol	386.368	428.384	.108746	9.19574

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[1] Acetyl number = 1335.39604 i.

Supplementary Notes.--The various hydroxy compounds that occur in oils, fats and waxes form derivatives on heating with acetic anhydride, the acetyl radical displacing the hydrogen of the alcoholic hydroxyl groups. This property serves as the basis of analytical methods for the quantitative determination of monohydroxy and dihydroxy acids and their glycerides, monoglycerides and diglycerides, and free alcohols.

Glycerides of hydroxy acids are a natural constituent of certain oils and fats although they do not appear to be very widely distributed in any considerable amount. Castor oil, composed largely of ricinolein, is an excellent illustration. Hydroxy acids probably occur more frequently as the result of oxidation of unsaturated acids. Oleic acid has been shown repeatedly to be comparatively unstable. By the assimilation of oxygen and water it may be converted into dihydroxystearic acid, a saturated compound.

$$C_{17}H_{33}COOH + H_2O + O = C_{17}H_{33}(OH)_2COOH$$

Whether the oxidation takes place in the glycerides or in the fatty acids after hydrolysis is uncertain although the latter appears the more probable supposition.

Monoglycerides and diglycerides result from the hydrolysis of triglycerides, and free fatty acids condition their presence; the absence of free fatty acids in a commercial product, however, does not necessarily preclude the presence of monoglycerides and diglycerides.

Solid alcohols of the cyclic series (sterols) occur in oils and fats both in combination as esters and as free alcohols.[1]

The amount of sitosterol or cholesterol is generally small, often inappreciable, and is indicated approximately by the unsaponifiable matter which it characterizes. Alcohols of the ethane and other series, free and in combination, compose a considerable proportion of waxes.

Oils and fats, therefore, may contain glycerides of monohydroxy and dihydroxy acids, possibly free hydroxy acids, monoglycerides and diglycerides and free alcohols; and the insoluble acids, separated from the oils and fats, may contain monohydroxy and dihydroxy acids and free alcohols. A portion, at least, of the free alcohols found in the insoluble acids probably occurred in the fat as esters. With the exclusion of the natural glycerides of hydroxy acids and a small amount of free alcohols, the acetyl number of many oils and fats may be deemed an index of quality, and when considered in conjunction with the acid and iodine numbers may serve to measure (more or less imperfectly, to be sure), the amount of hydrolysis and of oxidation the product has undergone. To differentiate between products of hydrolysis and of oxidation the percent of insoluble acids and their acetyl number should also be determined.

Of the oils, fats and waxes with an appreciable content of hydroxy compounds[2] might be mentioned candle nut oil, safflower oil, rape oil, peanut oil, olive oil, elderberry oil, Japanese sardine oil, skate liver oil, shark liver oil, seal oil, horses' foot oil, palm oil, bone fat and beeswax having acetyl numbers between 10 and 20, neat's-foot oil, Japan wax, carnauba wax and wool wax having acetyl numbers ranging from 25 to nearly 60, and castor oil having an acetyl number of about 170.

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[1] See numerous references: Abderhalden, Physiol. Chem. (1908); Hammarsten, Physiol. Chem. (1911); Leathes, The Fats (1910).

[2] Lewkowitsch, Analysis of Oils, Fats and Waxes, I, p. 434 (1913).

2. STABILITY OF BUTTER FAT SAMPLES.[1]

In the examination of butter fat, the question of stability is one of prime importance. Should appreciable changes take place in the samples, results would be vitiated and deductions as to the effect of feed would be of questionable value. That oils and fats are readily acted upon by a number of agents has been long recognized, but whether butter-fat samples as ordinarily treated would be sufficiently changed as to affect analytical results is uncertain, thought quite probable from the nature of the substance. To secure definite information on the subject it was necessary to carry out several experiments, of which a description with data follows.

The object of the first experiment was to determine the action of air, light and moisture, respectively, at the same temperature, upon butter fat. Heat as an independent factor could not be studied at that time as it would have increased the work to a point beyond which it could have been handled, but the action of heat was noted more particularly in another experiment. About 10 pounds of butter fat were prepared by melting fresh butter and filtering the supernatant fat through paper in a jacketed funnel. Two-ounce bottles, 73 in number, were filled with the melted fat and placed in the north window of the station creamery building in March, 1908. These bottles were divided into seven sets, four of which were closed with a glass stopper and sealed with ceresin to practically eliminate the oxidizing action of the air, and the remainder simply protected by a single thickness of unbleached cotton cloth tied over the top, which readily permitted circulation of the air. One set of the sealed bottles was guarded from light[1] and from moisture, and served as a check. Two sets of both closed and open bottles had 1 c.c. of water added, one set of each being exposed to north and east light (not sun) and one set protected from light[1]. Another set of both closed and open bottles was exposed to the light. From these various combinations it was thought deductions might be drawn as to the relative action of air, light and moisture upon butter fat.

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[1] This work was undertaken jointly with Dr. R. D. MacLaurin, but owing to the resignation of Dr. MacLaurin it has been completed and prepared for publication by the writer.

The fat was of fair average composition, as shown by the analytical results:

Saponification number,	232.47
Acid number,	1.48
Reichert-Meissl number,	29.84
Mean molecular weight of volatile acids,	96.90
Insoluble acids (per cent.),	88.21
Mean molecular weight of insoluble acids,	253.08
Iodine number,	28.40
Melting point (Wiley method),	32.95° C.
Refractive index, 40° C.,	1.4525
Valenta test,	28.50° C.

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[1] In providing for the circulation of air, a little diffused light reached the samples.

One or two samples were drawn from every series in June and December, 1908, and March and October, 1909, melted, filtered and analyzed. The testing in June, 1908, was more or less unsatisfactory, especially the iodine number, because of the high temperature prevailing, and what deductions may be offered will be based largely upon the remaining data, which represents periods of six, twelve and eighteen months.

Physical Changes.

The original fat, when melted, gave a transparent oil of a pronounced yellow color and a slight but characteristic odor. On standing, the color gradually faded. This, however, was far from uniform, even with members of the same series. The checks were very irregular, varying at the end of the test from yellow to almost white; with moisture the color was less intense, with light similar, and with moisture and light rather better than the checks. Light, in the absence of air, did not accelerate loss of color.

Air induced the most uniform destruction of color. As the air always carried more or less moisture, it was impossible to differentiate as to the effect of light and added moisture. The most notable change was obtained from the combined action of all three factors.

The sealed samples were porous, and developed a slight odor, unlike that of the original fat. The open samples were more like tallow, both in appearance and odor. Old samples containing added water were turbid on melting, and required considerable time to settle clear.

Chemical Changes.

As decomposition of fats and oil seems to progress along two fairly well-defined lines, that of hydrolysis and that of oxidation, only such determinations were planned as would readily serve to measure such changes; acid and saponification numbers for the former, and iodine number for the latter. Too much must not be expected of these determinations for so complex a reaction, but they are at least indicative. If the decomposition became extensive, other tests would be warranted.

As shown by Table I., added moisture, in the absence of air, had no appreciable hydrolytic action in excess of the check. Light alone, and with moisture present, preserved the original fat practically unchanged for eighteen months while the check manifested a noticeable breaking down. Moist air increased hydrolysis, both light and added water intensified the reaction. Lewkowitsch states that dry air without light has no action on oils and fats, and his explanation will be presented later.

Aldehydes were produced in both open and closed samples, as shown by the brown color of the saponification test (October, 1909), except in the sealed samples exposed to light.

Table I. Saponification Number.

	March, 1908.	June, 1908.		September,1908.		March,1909.		October, 1909.	
	Test.	Test.	Difference.	Test.	Difference.	Test.	Difference.	Test.	Difference.
Check, ...	232.47	233.32	+.75	232.93	+.46	232.81	+.34	234.28	+1.81
Moisture, ...	--	233.25	+.78	232.60	+.13	232.66	+.19	234.26	+1.79
Light, ...	--	233.20	+.73	232.33	-.14	232.33	-.14	231.99	-.48
Light and moisture, . .	--	232.80	+.33	232.53	+.06	232.75	+.28	232.36	-.11
Air and moisture, . . .	--	233.63	+1.16	232.97	+.50	235.19	+2.72	235.63	+3.16
Air and light, ...	--	234.24	+1.77	233.72	+1.25	235.98	+3.51	237.32	+4.85
Air, light and moisture,	--	234.51	+2.04	234.74	+2.27	238.21	+5.74	239.28	+6.81
Acid Number.									
Check, ...	1.46	1.40	-.06	1.51	+.05	1.42	-.04	2.75	+1.29
Moisture, ...	--	1.44	-.02	1.51	+.05	1.59	+.13	2.80	+1.34
Light, ...	--	1.40	-.06	1.40	-.06	1.39	-.07	1.60	+.14
Light and moisture, . .	--	1.41	-.05	1.44	-.02	1.59	+.13	1.93	+.47
Air and moisture, . . .	--	1.43	-.03	1.54	+.08	2.12	+.66	3.39	+1.93
Air and light, ...	--	1.48	+.02	1.57	+.11	2.29	+.83	4.03	+2.57
Air, light and moisture,	--	1.58	+.12	1.88	+.42	3.29	+1.83	5.90	+4.44
Ether Number.									
Check, ...	231.01	331.82	+.81	231.42	+.41	231.39	+.38	231.53	+.52
Moisture, ...	--	231.81	+.80	231.09	+.08	231.07	+.06	231.46	+.45
Light, ...	--	231.80	+.79	230.93	-.08	230.94	-.07	230.39	-.62
Light and moisture, . .	--	231.39	+.38	231.09	+.08	231.16	+.15	230.43	-.58
Air and moisture, . . .	--	232.20	+1.19	231.43	+.42	233.07	+2.06	232.24	+1.23
Air and light, ...	--	232.76	+1.75	232.15	+1.14	233.69	+2.68	233.29	+2.28
Air, light and moisture,	--	232.93	+1.92	232.86	+1.85	234,92	+3.91	233.38	+2.37

Table II. Iodine Number.

	March, 1908.	June, 1908.		September,1908.		March,1909.		October, 1909.	
	Test.	Test.	Difference.	Test.	Difference.	Test.	Difference.	Test.	Difference.
Check, ...	28.40	29.13	+.73	26.70	-1.70	27.88	-,52	25.88	-2.52
Moisture, ...	--	28.74	+.34	28.29	-.11	28.13	-.27	25.93	-2.47
Light, ...	--	28.26	-.14	28.08	-.32	27.38	-1.02	27.43	-.97
Light and moisture, . . .	--	26.86	-1.54	27.83	-.57	27.98	-.42	28.31	-.09
Air and moisture, ...	--	28.69	+.29	28.02	-.38	26.23	-2.17	25.42	-2.98
Air and light, ...	--	28.04	-.36	27.79	-.61	25.81	-2.59	24.66	-3.74
Air, light and moisture,	--	27.59	-.81	26.88	-1.52	24.43	-3.97	24.29	-4.11

In the absence of air, added moisture appeared to have no effect as compared with the check on the unsaturated compounds, while light both with and without moisture prevented oxidation to some extent as measured by the iodine number (Table II). The experiments of Ritsert[1] proved that light, in the absolute exclusion of air, could not produce rancidity, but the preserving action here noted is a peculiar feature worthy of further study.

Moist air increased the oxidation of the fat, with light and added moisture contributing factors. Light in the presence of moist air was destructive, a marked contrast to its action when air was excluded.

The hypothesis of Lewkowitsch[2], supported by the investigations of Geitel[3] and Duclaux[4], offers an explanation of the probable changes that take place in the development of rancidity in oils and fats. The initial change he ascribes to the action of moisture in the presence of fat-splitting enzymes. The free fatty acids resulting from the hydrolysis are oxidized by the air in the presence of light. Ritsert[1] asserts that oxygen and light must act simultaneously, neither of the agents alone being able to produce rancidity.

On the basis of the above assumption the hydrolysis of the check samples must have been due to traces of moisture in the fat and in the air between fat and stopper, and the oxidation to the air and a very limited amount of diffused light. This may be possible, as the changes were not, in themselves, excessive, though rather out of proportion to the conditions prevailing. It fails, however, to explain why similar samples in the light gave less rather than equal or greater changes under conditions which naturally should have been more favorable. The changes in the open samples were not wholly in accord with the theory. Light was a factor in oxidation, as was to be expected, but also in hydrolysis, which is difficult to explain. With many points indecisive and others unconsidered, the prime object of the experiment has been attained in showing that filtered butter-fat samples of normal acidity can be satisfactorily preserved in well-stoppered bottles. The action of high temperatures and sunlight should, of course, be avoided. As to the specific action of air, light and moisture, the experiment should be considered only preliminary, pointing the way for further work under "control" conditions.

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[1] Untersuchungen uber d. Ranzigwerden d. Fette. Inaug. Dissert. Berlin, 1890. (From Lewkowitsch).
[2] Analysis of Oils, Fats and Waxes, I, pp. 23-24 (1904).
[3] Jour. Prakt. Chem., 55, p. 448 (1897). (From Lewkowitsch).
[4] Ann. Inst. Pasteur, 1887; Compt. Rend. Acad. Sci., 102, 1077. (From Lewkowitsch).

Action of Heat.

The object of the second experiment was to ascertain what changes might take place upon heating butter fat several days at 50° C. Fresh samples were prepared. After heating a sample 24 hours in a water bath, varying amounts were weighed for saponification, acid and iodine numbers; similar portions were withdrawn at the end of 48 hours, and again after 72 hours' heating.

The analysis of the check sample and of the heated fat are presented in the following table:

	Saponification Number.	Acid Number.	Ether Number.	Iodine Number.
Check, ...	233.07	.84	232.23	28.18
Heated 24 hours, . .	233.99	.74	233.25	28.10
Heated 48 hours, . .	233.30	.81	232.49	28.17
Heated 72 hours, . .	233.62	.83	232.79	28.16

The results indicate a slight difference between the two samples in spite of careful mixing, as shown by the saponification and acid numbers. Heating gave a very slight increase in acid number, otherwise no change is noticeable. It seems evident, therefore, that any reasonable heating of butter fat at a temperature not exceeding 50° C. would have little apprecialbe effect upon analytical results.

3. PURIFICATION OF INSOLUBLE FATTY ACIDS.

Workers in oils and fats experience the same difficulty in obtaining chemically pure products as investigators in other lines of organic chemistry. The best insoluble fatty acids on the market--judging from our experience--are unsatisfactory in both physical characteristics and neutralization number. In general appearance the acids that are offered resemble granulated curd, though varying in color from white to yellow, and contain considerable dust and dirt. The molecular weight, as measured by titration[1] in an alcoholic solution, may deviate from the theoretical by 10 to 15 points. These statements apply to chemicals marked "C.P." and bearing the name of a reputable manufacturer or dealer.

The writer required stearic, palmitic, myristic, lauric and oleic acids for certain tests, and, finding it impossible to purchase them of the desired quality, was forced to undertake a study of various methods for their purification. As the character of the unsaturated acids is so unlike that of the saturated, only treatment of the latter will be considered at this time. The methods that seemed the best adapted for the purpose were distillation of the fatty acids in vacuo, crystallization from alcohol, and distillation of the tethyl esters in vacuo, and all were given extended trial.

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[1] The saponification process is preferable in some respects.

Distillation of the Fatty Acids in Vacuo.

Direct distillation under reduced pressure was successfully employed a few years ago by Partheil and Ferie[1], starting with Kahlbaum's best acids. Upon careful test the writer found that the method possessed certain objectionable features which render it rather impracticable for ordinary use. If it was merely a question of distillation of the acids, the process would be less difficult, but for fractionation, using a Bruehl or similar type apparatus, it proved almost impossible, in case of the higher acids, to prevent solidification in the side neck (outflow tube). The danger arising from a plugged apparatus at the high temperature involved has also to be taken into account. An attempt was made to heat the tube and keep the acids liquid by means of a hot-water jacket, also by an electrically heated asbestos covering, but neither process fully met the requirements of the case. The slow distribution of heat in vacuo is, of course, one of the obstacles in the way. For the distillation of solids of high melting point Bredt and A. van der Maaren-Jansen[2] devised an elaborate piece of apparatus having a flask and receiver of special construction, and an overflow tube heated by electricity, but it is hardly suited for a general laboratory or for handling any considerable quantity of material.

There are two other conditions necessary for a successful distillation of fatty acids, namely, absence of moisture and a current of hydrogen or carbon dioxide to prevent bumping and to lessen decomposition. Overlapping of the acids in different fractions cannot be obviated entirely, and if an unsaturated acid was present in the original, it will probably appear in nearly every fraction.

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[1] Arch. Pharm., 241, p. 545 (1903).
[2] Liebig's Ann. Chem., 354, p. 367 (1909).

Students under the direction of Professor Burrows of the University of Vermont have applied this process for a partial separation of the insoluble acids of several oils with a fair measure of success. With all due allowance for the possibilities of the method in the production of pure saturated fatty acids, the inherent difficulties render it inadvisable in most instances.

Crystallization from Alcohol.

Crystallization in this connection is practically limited in its application to the removal of a small amount of impurities, especially unsaturated acids. It can hardly be considered other than a supplementary treatment, though excellent for that purpose, to follow either of the distillation methods. Dry neutral alcohol suitable for such work can be prepared by distillation after treatment with caustic lime. In dissolving the acids, care should be taken to avoid heating to a higher temperature than is required for solution, or to prolong the heating unduly, as it will cause the formation of esters. Several minutes' boiling of the different fatty acids in alcohol caused the following loss in neutralization number:

Stearic acid, ...	1.70
Palmitic acid, ...	.56
Myristic acid, ...	2.24
Lauric acid, ...	.89
Oleic acid, ...	.28

Esterification undoubtedly causes a serious error by this process of purification. Under more careful treatment the change is not as rapid as shown above, but is evidently cumulative and may even exceed the figures given. Further study may warrant the substitution of a more stable solvent, such as acetone. For the filtration a water or ice jacketed funnel is almost necessary, particularly for the acids of low melting point, and suction is a time saver. Repeated crystallization is needed to bring out the true crystalline structure and silvery luster of the leaflet. Vacuum drying at a low temperature is one of the most efficient means for removing adhering alcohol and traces of moisture without injuring the structure. Crystallization as a whole is wasteful of acids and solvent unless both are recovered, but is essential for the production of a superior product.

Distillation of the Ethyl Esters in Vacuo.

As ethyl esters distill freely in vacuo, the process admits of a more ready application, and to products of a greater range of purity, than does a distillation of the acids. After considerable experimenting it was found that the esters are easily prepared by heating in an open flask equal parts (100 grams) of fatty acids and alcohol, together with a small quantity (10 c.c.) of concentrated hydrochloric acid, using capillary tubes to prevent bumping. The reaction requires about 30 minutes, after which the excess of hydrochloric acid can be removed with a separatory funnel. The distillation is conducted in a 500 c.c. "low" side neck flask, with a small (8 inch) Liebig condenser and a large size Bruehl fractionation apparatus. Heat is furnished by means of a linseed oil bath, and suction by a pump of any type, using a mercury manometer to prove constancy of vacuum. The neck of the flask from the shoulder to an inch or more above the side tube should be wound with asbestos paper to prevent cracking, due to sudden changes of temperature. The condenser should be kept full of water, without circulation, to serve as a hot-water jacket. The vacuum should be as high as the flask will safely withstand, but above all uniform, otherwise the fractions are of questionable value. The temperature range of an ester also varies with the distance between surface of liquid and side tube. At least one redistillation of like fractions is necessary.

As the esters are very stable, more difficulty was experienced in finding some means for their quantitative decomposition than in any other portion of the work. Heating with mineral acids hydrolyzes the esters very slowly, even under pressure. If, however, the esters are first saponified[1] by heating over a naked flame with twice their volume of glycerol and an excess of caustic potash until all the alcohol is expelled, and then the resulting soap dissolved in water and heated on a water bath with a slight excess of sulfuric acid, the separation is readily accomplished. This plan was suggested by the Leffmann-Beam saponification for volatile acids, and after extended trial proved the most thorough and rapid means for decomposing the esters. The resulting acid should be washed in a separatory funnel with boiling water until clear, and the cake allowed to drain. As previously stated, several crystallizations are necessary if a crystalline product of satisfactory melting point and neutralization number is to be secured. When crude acids are employed it is also advisable to crystallize at the outset to exclude a major part of the unsaturated acids, which otherwise would prove troublesome.

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[1] Observing the usual precautions given for the determination of insoluble fatty acids, Mass. Agr. Expt. Sta., Rpt. 21, p. 130 (1909).

To summarize:--saturated fatty acids may be purified by distillation of the acids or their ethyl esters. The latter method is less dangerous and easier to manipulate, although more steps are required. Crystalliaation is a finishing rather than an initial process of purification.

PURIFICATION OF OLEIC ACID.

The preparation of pure oleic acid by the methods in vogue is extremely difficult requiring long, tedious manipulation to insure the complete elimination of saturated (solid) fatty acids, acids of less saturation than oleic and neutral substances. Almost any of the nondrying oils or fats, such as almond oil, olive oil or tallow might be employed for the purpose but tallow is preferable, according to Lewkowitsch, as it is practically free from less saturated acids. Farnsteiner, however, obtained satisfactory results with olive oil. The prescribed treatment[1] consists in separating the liquid acids by solution of their lead salts in ether or benzene and purifying the resulting oleic acid by crystallization of the barium salt from alcohol or a mixture of benzene and alcohol. The method requires considerable time and practice and is necessarily rather expensive.

The experience gained in purifying saturated fatty acids warranted an attempt at similar treatment for oleic acid. The stock secured for experimental use had an iodine number of 84.08, equivalent to 93.49 percent oleic acid, which would indicate a grade of acid that is ordinarily prepared from tallow. Shaking out with boiling water had no apprecialbe effect, neither did centrifuging in the cold. As distillation at ordinary pressure would unquestionably result in serious decomposition and as distillation in a current of superheated steam was impracticable, distillation in vacuo was undertaken. The same apparatus was employed as in fractionating the ethyl esters of saturated acids except that a current of dry carbonic acid gas was substituted for air to facilitate distillation and to prevent decomposition. An ordinary suction pump failed to maintain as high or as constant a vacuum as desired, nevertheless a fraction was obtained between 218° and 223° C. which had an iodine number of 89.29, equivalent to 99.29 percent of oleic acid, which was very encouraging under the circumstances and promises a method worthy of further study.

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[1] For details, see Lewkowitsch, Analysis of Oils, Fats and Waxes, 1, p. 179 (1913).

4. AN ATTEMPT AT SEPARATING INSOLUBLE FATTY ACIDS BY THEIR LITHIUM SALTS.

The methods for fat analysis which have been described at some length are largely "group" tests. They may indicate the nature of an oleaginous product and, to some extent, the quality, in so far as hydrolysis and oxidation are concerned, but are insufficient for determining the various constituents, which seems essential when the effect of feed is being studied as in the case of butter fat. Numerous technical methods have been advocated for such work but few, if any, have received general acceptance. The insoluble acids merit first consideration as they comprise from 85 to 95 per cent of most oils and fats. A number of methods for the separation of the different insoluble acids is possible, namely:

Fractionation in vacuo of the fatty acids or their ethyl esters.

Fractional crystallization by reducing temperature and volume of solvent.

Solubility of their salts in organic solvents.

Fractional distillation as a means of purification has already been treated. From an analytical standpoint the process is objectionable in requiring complicated apparatus and too much material, in overlapping of fractions, in the occurrence of unsaturated acids in every fraction, and in decomposition. In brief, it is ill adapted for quantitative separations, a conclusion supported by Browne[1] and by unpublished data of the Chemical Department of the University of Vermont.

Crystallization is also beset with difficulties such as the maintenance of constant temperature during formation and filtration of crystals, slowness of formation, and overlapping. The method necessitates long tedious manipulation to insure results of value.

Salts of lead, magnesium, barium, lithium and other bases have been used by various workers in the separation of insoluble acids with some measure of success. Partheil and Ferie employed lithium and as their method[2] appeared the most promising it was adopted tentatively for the analysis of the insoluble acids of butter fat.

By their process 1 gram of sample is saponified with 15 c.c. of N/2 alcoholic potash, dissolved in 100 c.c. of 50 percent alcohol, neutralized with dilute acetic acid using phenolphthalein as indicator and a 10 percent solution of lithium acetate in 50 percent alcohol added. On heating to 60° C. the solution clears and, on cooling, lithium stearate, palmitate and a major portion of the myristate are said to precipitate leaving the remainder of the lithium myristate and lithium laurate, lithium oleate and less saturated acids in solution from which the unsaturated acids are separated by means of their lead salts. These fractions can be resolved into their constituent acids, dried, weighed and the mean molecular weight determined from which data the percentage of the different acids in each fraction can be readily calculated, provided no more than two undetermined acids are present in any one fraction. The value of the method depends in large measure on the correctness of the assumption that the various salts in mixtures of all proportions maintain solubilities conforming to the scheme of separation. The determined solubility of the several salts alone does not suffice.

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[1] Penn. State College Rpt. 1899-1900, p. 228 (1900).

[2] Arch. Pharm. 241, pp. 545-569 (1903).

After considerable preliminary work it was deemed advisable to begin operations with 1 gram of insoluble acids instead of fat, removing the soluble acids and glycerol before, rather than during the process, and thereby eliminate the possible influence of these factors which in the case of butter fat constitute a considerable percentage. The strength of alcohol, ratio of fatty acids to solvent and temperature of solvent during crystallization were studied with the result that 1 gram to 150 c.c. of 50 percent alcohol at 17° C. were adopted as apparently the most satisfactory from the standpoint of practical manipulation. The acids are converted into soaps by heating to 60° C. and titrating with N/2 alkali, using phenolphthalein as indicator. The amount of precipitant and conditions favoring the formation of a crystalline precipitate were also carefully investigated. Lithium has weak basic properties. Three times the calculated amount of lithium acetate dissolved in 50 percent alcohol (15 to 100) added to the warm soap solution after faintly acidulating with dilute acetic acid, throws down on cooling[1] a bulky semitransparent, granular precipitate of salts of the higher fatty acids that filter and wash readily. These conditions entirely prevent the formation of sticky precipitates, difficult or impossible to handle. The high solubility of the precipitated salts necessitates rapid filtration with a minimum of washing in order to avoid serious loss. This was finally accomplished by means of a filter tube with a cotton felt supported by a glass bead using suction. The washing was limited to four successive 5 c.c. portions of alcoholic-acetate wash (1 gram acetate to 100 c.c. 50 percent alcohol), and is effective on the small area involved. The wash was based on the theory of like ions, analogous to the treatment of Paris green and is preferable to either 50 percent alcohol or water. The precipitate is washed back into the original flask with boiling water and the alcohol carefully evaporated from both fractions in a water bath. Partheil and Ferie convert the lithium salts of the filtrate into lead salts and separate the unsaturated from the saturated acids by the lead-salt-ether method. A careful investigation indicated that this separation was superfluous where only one unsaturated acid is present, as it could be readily determined later by the iodine process. With this modification the alcohol-free fractions are made to a volume of 150 c.c. with hot water, 0.5 gram of ceresine added to coalesce the fatty acids and to lessen decomposition of unsaturated acids and treated with a slight excess of sulfuric acid to free the fatty acids which are washed, dried in a vacuum oven at 60° C. and weighed in a manner similar to that prescribed for insoluble acids.

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[1] Frequent rotation of the solution is necessary during the coolin

Solution of the separated fatty acids in alcohol and titration with alkali, as in acid number, did not yield accurate molecular weights, due possibly to the formation of anhydrides or of ethyl esters, to the presence of ceresine or to the reluctance with which oleic acid combines with its full amount of alkali. Saponification with an excess of alcoholic potash proved more satisfactory

After titrating, the solution is rendered distinctly alkaline, the alcohol evaporated and the unsaturated acids in both fractions determined on the dry residues by the iodine absorption, Wijs solution. The accuracy of the iodine process for ordinary oleic acid is conceded and it certainly is preferable to the lead-salt-ether method for this purpose.

The Partheil and Ferie method was observed in all essentials and improved in many particulars, but the solubility of the various lithium salts did not conform to the promulgated scheme of separation; for instance, about one-quarter of the lithium oleate (the most soluble salt) was precipitated in nearly every case. Work on the Partheil and Ferie method was undertaken after a careful review of their report and with the expectation that the process fully met the claims set forth. The investigation was continued about three years, except during hot weather, and many difficult features of manipulation solved, but changes in detail could not rectify errors in principle.

The total recovery of fatty acids was excellent, generally exceeding 95 percent. The final results or calculated percentages of the several fatty acids, however, were not sufficiently concordant nor of a character to warrant further work along this line. This failure was due, in our judgment, to inherent faults of the method and not to manipulation. The reasons for the same may be briefly summarized.

Failure of the salts to maintain the solubilities claimed.

The amount of material that can be handled by the method is inadequate for satisfactory molecular weight determinations which are an essential part of the test.

The one-gram portion of fatty acids under examination is subjected to four distinct processes (three of which come after its division into two parts) requiring 8 to 10 days for completion. To obtain *quantitative* results on so unstable a product and in so small an amount under such treatment necessitates an *exactness* greater than analytical procedure permits.

5. DETERMINATION OF STEARIC ACID IN BUTTER FAT.

Introduction.

Oils and fats are composed largely of neutral glycyl esters together with small amounts of free fatty acids and unsaponifiable matter. Formerly the esters were considered simple glycerides, compounds of glycerol and three radicals of the same fatty acids. At present the opposite view seems to prevail and mixed glycerides are said to predominate in most products. The subject is controversial and difficult of solution. The constituents would be the same, however, in either case whether combined as simple or complex molecules. The object of a technical examination of oils and fats is to isolate, identify and determine the various fatty acids, glycerol and unsaponifiable bodies although, as Lewkowitsch asserts, this is not attainable in the present state of our knowledge. Certain progress has been made in determining different constituents of fats by indirect methods such as the iodine absorption, acetyl number, molecular weight calculations, etc. Direct methods of fractional distillation, crystallization and solubility of various salts have not, as a rule[1], proved sufficiently discriminative for quantitative use.

After several years' investigations of the Partheil and Ferie method[2] which proved unsatisfactory in our hands, the author undertook a study of methods for determining stearic acid in butter fat.

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[1] There are some exceptions, however.
[2] Arch. Pharm., 241, pp. 545-569 (1903).

Earlier Investigations.

For the separation of stearic from other fatty acids, David recommended[1] a special alcohol and dilute acetic acid solution saturated with stearic acid at 15° C., in which solution oleic acid was shown to be soluble.

The Hehner and Mitchell method[2] for isolating stearic from other fatty acids was based on the hypothesis that a mixture of fatty acids heated with a solvent saturated at a given temperature with the acid under determination, might be expected on cooling to that temperature to crystallize the whole of the acid sought, provided the other constituents did not increase the solubility. The solvent employed was methylated alcohol (94.4 percent) saturated with stearic acid at 0.2° C., prepared by chilling.a solution of 3 grams to a liter overnight in ice water, and siphoning off the saturated mother liquor through a small thistle tube covered with fine calico, using suction. The tests were conducted in a similar manner, taking from 0.5 to 5 grams of insoluble acids (according to content) to 100 c.c. of alcoholic-stearic solution. Shaking was found to increase precipitation. Supersaturation and esterification were recognized as possible sources of error. The method gave concordant results with solid fats containing considerable stearic acid, but slight, if any, precipitate from the acids of butter fat and from mixtures of the acids of Japan wax and pure stearic acid.

Emerson noted[3] considerable variation in the content of different saturated solutions and found that supersaturation seemed to occur when less than 0.7 gram to 100 c.c. was employed in preparing the solution. The formation of ethyl esters appeared to be a souce of error and to have increased the apparent solubility of the stearic acid.

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[1] Compt. Rend. Acad. Sci., 87, pp. 1416-1418. Jour. Chem. Soc. Abs. 34, pp. 1011-1012 (1878).

[2] Analyst, 21, pp. 316-331 (1896).

[3] Jour. Amer. Chem. Soc. 29, pp. 1751-1756 (1907).

Kreis and Hafner showed[1] that small amounts of stearic acid below 0.1 gram to 100 c.c. of a saturated solution, formed supersaturated solutions, and that less than 0.05 gram gave low and extremely variable results even upon the addition of crystals of stearic acid.

Lewkowitsch claimed[2] that the method yielded capricious results with mixtures of stearic, palmitic, and oleic acids, and that in many cases the results were entirely unreliable when other acids were present. He stated that a considerable proportion of lauric acid would prevent the complete precipitation of stearic acid even when supersaturated alcoholic-stearic solutions were used, and that acids of higher melting point, when present, such as arachic, dihydroxystearic, etc., would appear in the separated acids. He reported a precipitate of 0.49 percent from butter fat, of which a portion might be arachic and myristic acids.

The results obtained by various investigators indicate that the solubility of stearic acid increased with the strength of the alcohol, but the figures reported are too variable to warrant further deductions.

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[1] Ztschr. Untersuch. Nahr. u. Genussmtl., 6, pp. 22-27 (1903).
[2] Analysis of Oils, Fats and Waxes, 1, pp. 556-559 (1913).

Solubility of Stearic Acid.

	Approximate Strength of Alcohol. (Percent).	Stearic Acid to 100 c.c. (Grams).	Saturation of 100 c.c. at 0° C. (Grams).
Hehner and Mitchell	94.4	0.2 - 0.5	.1400-.1580
Emerson	95.5	.7	.1223
"	95	.7	.1123
"	94.5	.7	.1035
Kreis and Hafner	95.	.5	.1220-.1310
Lewkowitsch[1]	94.4	.3	.0814
"	94.4	.7	.0810-.1082
Ruttan[2]	100.	-	.373

[1] Loco citato, p. 164.

[2] Eighth Internat. Cong. Appl. Chem., 25, p. 440 (1912).

Preliminary Work.

In view of what has been stated, the outlook for another investigation was not promising although Lewkowitsch's final arraignment of the process was not published until nearly a year after the work was undertaken. The subject was of sufficient importance, however, to warrant additional study, whatever the outcome.

Apparatus.--To insure uniform temperature for crystallization, a tank was constructed of 7/8" material (20 1/2" long, 10 1/4" wide and 20 1/2" deep), lined with galvanized iron, provided with a tight cover and supported by legs to a convenient working height. For icing, a basket (13 1/2" x 6" x 18") of galvanized screening of 5/16 mesh, holding probably 30 pounds of broken ice, was found very satisfactory. The insulation with wood together with the large volume of water and ice proved inadequate to meet the requirements of the case and it was necessary to install in one corner of the tank a pump run by a motor, to keep the water in continuous circulation. With this apparatus a constant temperature of about 0.1° C. was easily maintained.

Several factors had to be considered in the selection of containers in which the tests were to be conducted. They must be of a form, size and weight suitable for weighing the charge on analytical balances, easily held in position in the tank and from which the alcoholic solution could be removed while still in the tank, leaving the crystalline residue. After numerous experiments with globe-shaped separatory funnels and filtering tubes, 8-ounce sterilizer bottles[1] were adopted and have been found entirely satisfactory. The bottles are of narrow cylindrical form (6 3/4" x 2") and are held in place in the tank by pockets of wire screening with only the rubber stopper and a small portion of the neck projecting out of the water. The solution is siphoned off by means of a small thistle tube (1/4" bulb) having a felt of absorbent cotton weighing .015 gram supported by a glass bead, and covered with a piece of cheesecloth.

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1 Manufactured by Whitall Tatum Company.

Reagents.--For the preparation of an alcoholic-stearic solution, consittuents of high quality were deemed essential for satisfactory work. The purification of alcohol had been a subject for study for a number of years in connection with the ordinary analysis of oils and fats, as previously described under "Improved Methods for Fat Analysis". The strength averaged over 95 percent by volume, although the lime employed contained too much hydrate to be efficient as a drying agent.

One lot of stearic acid, a mixture of several grades, was purified by fractional distillation of the ethyl ester in vacuo and subsequent repeated crystallization of the separated acids from alcohol as already described under "Purification of Insoluble Fatty Acids". Another lot of acid with a molecular weight of 271.13 was purified by 10 or more crystallizations from alcohol to a molecular weight of 284.25, and again to 284.71, although the resulting leaflets were far less perfect than in the former case.

When using separatory funnels and filtering tubes, alcoholic-stearic solutions, saturated at 0.1° C., applied to the insoluble acids of butter at the rate of 150 c.c. to 0.5 gram of material seldom yielded an appreciable amount of precipitate on standing even with the addition of crystals of stearic acid and thorough agitation. Solutions testing about 0.22 and 0.24 gram of stearic acid to 150 c.c. gave somewhat higher results, although of erratic and untrustworthy character. In the attempt to develop a method with this apparatus, over 140 determinations were made on butter acids, stearic acid mixtures of butter and stearic acids, stearic and oleic acids and stearic, myristic and oleic acids. The object was not attained, and most of the data will be omitted as it would serve no useful purpose, merely indicating the time and labor involved. The results, however, with solutions of stearic acid alone appear to warrant certain deductions:

Solutions containing from 0.25 to 0.29 gram of stearic acid to 150 c.c. crystallized, leaving mother liquor of unlike composition (saturation).

Saturation varied inversely with the amount of stearic acid present.

Presumably, therefore, supersaturation occurred as a result of insufficient stearic acid. See table following.

Time of standing may have had some influence but when in excess of 24 hours was of minor consequence.

The form of the container as viewed in the light of subsequent work was a factor of considerable importance; a globe-shaped vessel was less effective than a long, narrow, cylindrical one of large surface area.

Crystallization of Stearic Acid from Solutions of Different Content.

(Using Separatory Funnels).

Alcoholic-Stearic Solution. (Grams in 150 c.c.)	Additional Stearic Acid Taken. (Grams).	Precipitate. (Grams).	Saturation. (Grams in 100 c.c.).
.2406	.0100	.0130	.1584
.2406	.0150	.0254	.1535
.2406	.0150	.0315	.1494
.2406	.0400	.0859	.1298
.2406	.0450	.0995	.1241
.2400	.0200	.0426	.1449
.2400	.0251	.0544	.1405
.2400	.0304	.0640	.1376
.2400	.0354	.0733	.1347
.2400	.0475	.0872	.1335
.2400	.0481	.0910	.1314
.2400	.0491	.0910	.1321
.2400	.0498	.0960	.1292

Stearic acid solutions were found to crystallize more readily and with greater uniformity in sterilizer bottles than in separatory funnels, probably due to the more rapid chilling of the narrow column of liquid and more thorough filtration.

The following table shows the amount of stearic acid crystallized from solutions of different content and the saturation of the mother liquor.

Crystallization of Stearic Acid from Solutions of Different Content.

(Using Sterilizer Bottles).

Alcohol. (c.c.)	Stearic Acid Taken. (Grams)	Precipitate. (Grams)	Saturation. (Grams in 100 c.c.).
150	.2000	.0000	--
	.2400	.0020	.1587
	.2705	.0485	.1480
	.2815	.0700	.1410
	.3055	.1110	.1297
	.3215	.1280	.1290
	.3475	.1680	.1197
	.3600	.1815	.1190
	.3670	.1880	.1193
	.3800	.2000	.1200
	.4000	.2210	.1193
	.4080	.2260	.1213
	.4200	.2435	.1177
	.4650	.2980	.1113
	.5000	.3255	.1163
	.6000	.4315	.1123

Alcoholic-Stearic Solution (3990 grams in 150 c.c.)	Alcohol. (c.c.)	Equivalent in Stearic Acid. (Grams in 150 c.c.)	Precipitate. (Grams)	Saturation. (Grams in 100 c.c.)
100	50	.2660	.0555	.1403
110	40	.2926	.0980	.1297
120	30	.3192	.1500	.1128
130	20	.3458	.1745	.1142
140	10	.3724	.2055	.1113
150	0	.3990	.2335	.1103

Application.

The facility with which alcoholic-stearic solutions crystallize increased with the concentration. Solutions of 0.38 to 0.40 grams to 150 c.c. formed crystals readily, gave a satisfactory amount of precipitate and when applied to the insoluble acids of butter yielded an additional amount from that source. This would indicate that if the stearic acid of the solution is sufficient, crystallization of stearic from butter acids is no more difficult than from other products. The results were very concordant for a crystallization method when all details of manipulation were strictly observed; the water maintained at the required level, properly iced at all times and the pump run continuously at good speed. A gentle rotation of the solution after standing overnight in the ice tank assisted in completing the precipitation, but anything in the nature of shaking reduced the fragile crystals to a mass and rendered filtration extremely difficult or impossible.

Method in Detail.

Five-tenths (0.50) of a gram of melted insoluble acids are brought into an 8 ounce sterilizer bottle and 150 c.c. of an alcoholic-stearic solution (2.67 grams to 1000 c.c.), accurately measured with a pipette, added. It is considered advisable, though possibly unnecessary, to allow the charge to stand overnight in order to regain its normal crystalline structure as required in melting point determinations before adding the solvent. The bottle is sealed with a solid rubber stopper, shaken at room temperature until the charge is completely dissolved, placed in a pocket of the ice tank and allowed to stand overnight. The following morning the solution is gently rotated and in the afternoon is siphoned off as thoroughly as possible by means of a small thistle tube[1] and a perforated rubber stopper, using suction. The residue is dissolved in ethyl ether, transferred to a tared 140 c.c. wide mouth Erlenmeyer flask, the ether carefully distilled off, dried at 100° C. and weighed. As saturation is likely to vary more or less with the amount of stearic acid present, and as the quantity of solution retained by the precipitate depends in a measure on the amount of precipitate, blanks are run on a weight of stearic acid equivalent to that expected in the test. By deducting the stearic acid taken, from the weight recovered, the true blank for the alcoholic-stearic solution is obtained.

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[1] The size of the tube and preparation of the felt has already been described.

Nature of the Precipitate.

To ascertain whether the crystalline substance obtained from butter acids was stearic acid or a mixture, the residues from a number of tests (one being insufficient for accurate work) were combined and the molecular weight determined by saponification. The last of these determinations made after considerable experience with the stearic acid method gave 284.64, theory 284.288. The melting point was not determined as it was considered less reliable than the molecular weight.

Influence of Different Fatty Acids on Precipitation of Stearic Acid.

Numerous tests were made in an effort to determine whether lauric, myristic, palmitic and oleic acids had any effect on the crystallization of stearic acid and if so, the nature and extent of such action. The following table will serve to illustrate.

Effect of Different Fatty Acids.

Alcoholic-Stearic Solution. (Grams in 150 c.c.)	Additional Stearic Acid Taken. (Grams)	Other Acids Taken. (Grams)	Precipitate. (Grams)	Saturation. (Grams in 100 c.c.)
.3990	.1000	--	.3420	.1047
	.1015	--	.3430	.1050
	.1035	--	.3415	.1073
	.1000	--	.3405	.1057
		Lauric.		
	.1030	.4000	.3455	.1043
	.1000	.4000	.3415	.1050
	.1000	.4000	.3430	.1040
	.1010	.4000	.3450	.1033
		Myristic.		
	.1000	.4000	.3495	.0997
	.1010	.4000	.3480	.1013
	.1000	.4000	.3490	.1000
	.1000	.4000	.3515	.0983
		Palmitic.		
	.1055	.4000	.3135	.1273
	.1000	.4030	.2980	.1340
	.1010	.2500	.2965	.1357
	.1040	.2500	.3065	.1310
	.1050	.2000	.3085	.1303
		Oleic.		
	.1070	.4220	.3515	.1030
	.1010	.4255	.3440	.1040
	.1000	.4000	.3485	.1003
	.1035	.4000	.3460	.1043

According to molecular weight determinations, the lauric and palmitic acids were of excellent quality and the myristic and oleic acids, a little inferior.

Lauric, myristic and oleic acids in relatively large amounts showed no appreciable influence on crystallization of stearic acid. Palmitic acid, on the other hand, noticeably increased the solubility and changed the physical character of the precipitate. The subject will be given further study with a view of counteracting the restraining influence of palmitic acid, or at least reducing it to a minimum.

Results obtained with the Insoluble Acids of Different Butters.

Station Number.	Stearic Acid. (Percent).
1	10.17
2	8.84
6	6.93
8	8.27
10	7.60
11	17.25
12	8.56
13	14.94
14	10.10
16	9.03
17	13.87

The presence of relatively large amounts of palmitic acid in prepared mixtures affected the crystalline structure of the precipitate. The addition of palmitic acid to butter acids materially increased the solubility, thereby confirming similar tests with stearic and palmitic acids already cited. Some of our latest determinations indicate that the action of palmitic acid can be counteracted by increasing the relative amount of stearic acid.

Summary.

The method proposed for the determination of stearic acid in the insoluble acids of butter fat is a direct crystallization from a definite supersaturated alcoholic-stearic solution under control conditions. The process is simple, requires only a single reagent and no difficult manipulation. The results while very much higher than those generally reported, are concordant and molecular weight determinations leave no doubt as to the character of the precipitate.

RESUME.

The most important results secured from the preceding investigations are as follows:

1. Improved Methods for Fat Analysis.

The standardization of the various "group" methods for the analysis of oils and fats promotes accuracy and insures more comparable data by greater uniformity and simplicity of methods embracing definition of terms, principles involved and details of manipulation including reagents, apparatus and glassware. Among the prominent features treated might be mentioned amount of material employed, strength, preparation and quantity of reagents and indicators, numerous improvements in the various processes, synopsis of reactions, and limits of error. Particular attention is called to the synopsis of composition which presents the aim of a technical analysis although not fully attainable in the present state of our knowledge.

2. Stability of Butter Fat Samples.

Filtered butter fat of normal acidity is shown to be a fairly stable product and considerable light is thrown on hydrolytic and oxidation changes as a result of the action of air, light, moisture and heat respectively.

3. Purification of Insoluble Fatty Acids and Purification of Oleic Acid.

Saturated fatty acids can be purified by fractionation of the acids or their ethyl esters in vacuo. The latter process is shown to be easier to manipulate although requiring more steps.

Crystallization is a finishing rather than an initial process of treatment. Fractional distillation of oleic acid in vacuo in a current of carbonic acid gas promises to be a satisfactory method of purification.

4. An Attempt at Separating Fatty Acids by their Lithium Salts.

The separation of insoluble fatty acids by means of their lithium salts proved unsatisfactory, due to inherent faults of the method arising from failure of the salts to maintain the solubilities claimed and from the inadequate amount of material that could be handled.

5. Determination of Stearic Acid in Butter Fat.

A method was adapted for direct crystallization of stearic acid in the insoluble acids of butter fat from a definite supersaturated alcoholic-stearic solution under control conditions. The process is simple, requires only a single reagent, and no difficult manipulation, and gives concordant results very much higher than those generally obtained.